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JOURNAL OF THE NATIONAL CANCER INSTITUTE

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2. All patients registered on study should be accounted for. The report should specify for each treatment the number of patients who were not eligible, who died or withdrew before treatment began. The distribution of follow-up times should be described for each treatment, and the number of patients lost to follow-up should be given.

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4. In randomized studies, the report should include a comparison of survival and/or other major endpoints for all eligible patients as randomized, that is, with no exclusions other than those not meeting eligibility criteria.

5. The sample size should be sufficient to either establish or conclusively rule out the existence of effects of clinically meaningful magnitude. For "negative" results in therapeutic comparisons, the adequacy of sample size should be demonstrated by either presenting confidence limits for true treatment differences or calculating statistical power for detecting differences. For uncontrolled phase II studies, a procedure should be in place to prevent the accrual of an inappropriately large number of patients, when the study has shown the agent to be inactive.

6. Authors should state whether there was an initial target sample size, and, if so, what it was. They should specify how frequently interim analyses were performed and how the decisions to stop accrual and report results were arrived at.

7. All claims of therapeutic efficacy should be based upon explicit comparison with a specific control group, except in special circumstances where each patient is his own control. If nonrandomized controls are used, the characteristics of the patients should be present in detail and compared to those of the experimental group. Potential sources of bias should be adequately discussed. Comparison of survival between responders and nonresponders does not establish efficacy and should not generally be included. Reports of phase II trials which draw conclusions about antitumor activity but not therapeutic efficacy generally do not require a control group.

8. The patients studied should be adequately described. Applicability of conclusions to other patients should be carefully dealt with. Claims of subset-specific treatment differences must be carefully documented statistically as more than the random results of multiple subset analyses.

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**From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69: 1-3, 1985).

Cancer Treatment Reports

National Cancer Institute, Vincent T. DeVita, Jr, ***Director***

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EDITORIAL

Identification of New Drugs in Small Cell Lung Cancer: Phase II Agents First?[1]

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There has been a considerable proliferation of literature regarding the treatment of small cell lung cancer, with a large number of reports clearly testifying to the role of chemotherapy in producing response, prolonging median duration of survival, and achieving long-term survival in a few patients with limited disease (1). For patients with extensive disease, the prognostic situation is considerably more adverse. The complete response frequency is considerably lower and, in most series, less than one-half of that in limited disease. The median duration of survival, approximately 9 months, is modest, and there are virtually no long-term survivors; the vast majority of patients die within 2 years (2). Currently, chemotherapy has had a substantial impact on this disease in that the median duration of survival has shifted from 6 weeks to nearly 9 months. These modest improvements have appropriately led to reappraisal of our treatment strategy for patients with extensive small cell lung cancer. In particular, the philosophy of testing new agents has come under scrutiny because of the lack of ability to identify new active agents in this disease. In a review of 97 phase II trials in small cell lung cancer, Cohen et al (3) showed that the response frequency was $< 20\%$ for most agents, and for the few agents that were adequately tested in both previously treated and untreated patients, the response rate was higher in previously untreated disease. This is strong testimony to the difficulty in identifying new agents in previously treated disease.

Small cell lung cancer is thus the human model of pleiotropic drug resistance. With only minor exceptions (4,5), second-line chemotherapy in small cell lung cancer is usually not effective in producing major response or prolongation of survival. The response frequencies to second-line chemotherapy are considerably lower than those with first-line agents, and the median duration of survival after relapse is usually ≤ 3 months. This can be well exemplified by the use of etoposide in small cell lung cancer. Etoposide used as a single agent is highly active in this disease and can, depending on dose and schedule, produce a response rate of approximately 45% in previously untreated disease (6). When tested in patients given previous combination chemotherapy, however, the response for etoposide is $< 10\%$ (7). There are some reports of active second-line combinations (4,5), but these combinations are rarely as effective as when they are used as first-line treatment. One exception to this is the sequence of two specific combinations, ie, etoposide plus cisplatin following the combination of cyclophosphamide, doxorubicin, and vincristine (4). Second-line responses are, however, not usually seen when the combinations are given in reverse order. A number of investigators realized some years ago that our strategy for the identification of new drugs in small cell lung cancer must be changed; otherwise, potentially active agents such as etoposide would be missed in screening previously treated patients. A number of such strategies have been proposed, and each strategy offers potential difficulties in either utilization of resources, protocol design, or ethical issues.

One approach for screening new compounds would be to include a new agent as part of a randomized study in combination with other agents. The difficulty with this approach is that it may provide only minimal information about the single-agent activity in small cell lung cancer. The more active the combination in which the new agent is utilized, the less likely we are to see an incremental effect. This approach will guarantee the least efficient utilization of the patient resources. Testing of the effect on survival would require accrual of relatively large numbers of patients in each arm. The effect on total response would also be difficult to assess since the total response rate, even in extensive disease, is high. The effect on complete response may produce some useful information, but again, this would have to be considered in the light of other agents used.

Another approach might be to lower our threshold of acceptability below 20%, the usual threshold for determining activity in phase II trials. The difficulties with this approach are multiple. A larger number of patients

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would have to be treated to gain sufficient confidence (90% or 95%) that the true response rate was less than some lower threshold such as 10%. Thus, instead of excluding a 20% level of activity with 90% or 95% confidence (no response for 15 patients or for 19 patients, respectively), one would exclude a 10% level of response with 90% or 95% confidence and would therefore raise the number of patients potentially given ineffective therapy (0 for 22 patients or 0 for 29, respectively) (8). This raises some potential objection in regard to offering therapy with low probability of response to patients with inevitably fatal disease. This is especially true when such agents have potential toxic effects and can impact negatively on the quality of the remaining survival. In this situation, however, the treatment alternatives are minimal, and such an approach, particularly in a cooperative group in which the rapid accrual will prevent a "no response fatigue" on accrual, would be very acceptable. Using such an approach, cisplatin probably would have been accepted but etoposide may not have been accepted.

Another approach to new drug testing in small cell lung cancer might be to test new agents after the patients have received initial therapy and achieved a stable partial response, eg, after three courses. Although this approach is appealing because it offers the patient primary treatment benefit, it has several possible confounding problems. First, the tumor will have been exposed to prior chemotherapy, making the evaluation of any additional response subject to the same criticism as when second-line therapy is tested after primary combination drug failure. Second, any impact beyond augmenting initial response would be lost without the use of a randomized trial.

Yet another approach to this problem has been to offer phase II agents as initial therapy for patients with extensive disease. The rationale offered by this approach is that patients with good performance status but extensive disease have a poor probability of response, a relatively short median survival, and essentially no chance of long-term survival; there is also an important need for identifying new active agents. Implicit in this approach is one of two concepts: that initial treatment with a phase II agent is unlikely to have an adverse effect on subsequent responsiveness or that the overall survival probability is essentially unchanged from sequential drug applications. Can one justify the use of phase II agents in initial treatment as a routine screening method the way one applies phase II agents in other diseases for which there is no "effective" therapy? Clearly, this approach would maximize the ability to find new potentially active agents. Several new agents have been identified using this approach, including teniposide and carboplatin (9–11), but both also have some activity in second-line therapy.

Several concepts should be considered, however, if this approach is utilized as a method for new drug identification for small cell lung cancer. First, dose is a critically important factor in the treatment of small cell lung cancer. Virtually every study that has tested the concept of dose has shown a dose-response relationship. Studies in which dose is ameliorated have produced fewer complete responses and shorter median survival. In randomized studies, either the response frequency, or the number of long-term survivors, or both have been better in the high-dose arm. Thus, the application of phase II agents in less than a maximally tolerated dose as determined by the biologic effect on specific targets such as wbc and platelet count nadirs is a suboptimal test of any agent. Rather than use the lower end of a maximal tolerated dose range identified in phase I testing, investigators seeking to test new phase II agents should apply the highest tolerated dose, ie, a dose that will produce sufficient myelosuppression to be comparable to that achieved with combination chemotherapy.

Next, careful attention should be paid to the known pharmacology of a given agent. When schedule is an important factor, this must be taken into consideration. For example, if etoposide were to be tested in phase II today, the best test would be a multiple-day schedule such as treatment on days 1–5 or days 1–3, rather than once every 3 weeks. Similarly, a drug such as diaziquone, which has previously been shown to be inactive in recurrent disease (12), might be tested by continuous infusion (13).

Third, it is critically important that if investigators choose to utilize phase II agents as first-line treatment, the subsequent response to combination chemotherapy should be included as part of the report. In a disease as highly responsive as small cell lung cancer, the diminution of response by a phase II agent may be of critical importance in determining subsequent drug-testing strategy. Similarly, the survival experience for the entire group treated initially with a phase II agent, although not usually included as part of a phase II trial, may bear important information regarding the overall experience of the treated group.

One modification of this approach offers some possibilities in terms of new drug testing in small cell lung cancer. A number of well-characterized cell lines derived from both treated and untreated human samples are now readily available. These cell lines could be used as a pretreatment screen to define which phase II agents would offer the possibility of producing responses in patients with extensive small cell lung cancer. Thus, studies of the activity of new agents in these cell lines would offer some insight into the response potential of these agents. Lacking such information, one might have considerable hesitancy to test new agents because of the potential to interfere with the subsequent response to and the palliative capacity of combination chemotherapy.

Several recently completed phase II studies in extensive small cell lung cancer show adverse consequences (14–17). In each of these studies, there was a low initial response rate, and the subsequent response and survival were apparently impaired. The median duration of survival in these extensive disease groups was ≤ 6 months;

it was apparently worse than that previously experienced at each institution. Furthermore, the study by Lund et al (16) at the Finsen Institute was based on an agent that was highly active in cell lines. These studies suggest that this treatment strategy, ie, using phase II agents as initial treatment, may not be an adequate approach. There is currently a randomized study by the Eastern Cooperative Oncology Group (ECOG) that addresses this issue directly in a randomized phase II study of a new agent followed by combination chemotherapy vs combination chemotherapy alone. If the results of this trial were to show that there is indeed no profound adverse effect on the survival of the group as a whole, then this approach would still be justified. However, the available results of the phase II trials argue against this approach, and further trials using this philosophy should await the results of the ECOG trial. If, on the other hand, the ECOG study, like the phase II studies, were to show that subsequent responsiveness and survival were significantly impaired by the application of new agents, we would have to once again reappraise our treatment strategies for the identification of new agents and choose from among the other options. Currently, the most preferable choice is the lowering of our level of acceptance to 10% in previously treated patients.

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Distribution of Clomesone in Mice[1,2]

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The distribution of the novel alkylating agent clomesone [2-chloroethyl (methylsulfonyl)-methane sulfonate] has been studied in mice after iv administration of 35 mg/kg. In plasma, [^{14}C]clomesone was eliminated in an apparent single phase with a half-life of 11 minutes. The elimination of total radioactivity occurred with an initial half-life of 51 minutes, and then in a prolonged phase of undetermined length. At least two organic soluble metabolites were detectable in plasma. Highest tissue concentrations of radioactivity were in liver, kidney, and small intestines where, at most times of analysis, the levels were two to three times higher than those in plasma. The rate of elimination of radiolabel from spleen, lungs, brain, and muscle, which contained about equal concentrations of radioactivity at most times after dosing, paralleled the rate of loss from plasma. A major proportion of the radioactivity in tissues was bound to trichloroacetic acid-precipitable macromolecules. Within 24 hours, 77.6% of the dose was recovered in urine. High-pressure liquid chromatographic analyses of 8-hour urine collections demonstrated that < 2% of the dose was excreted unchanged. These results demonstrate that clomesone is rapidly eliminated from plasma and that it and/or its products become extensively bound to tissue components. [Cancer Treat Rep 71:1135–1140, 1987]

Of the anticancer agents currently in clinical use, alkylating agents that display chloroethylating activity are among the most effective. BIC (5-[3,3-bis(2-chloroethyl)-1-triazenyl]-1*H*-imidazole-4-carboxamide), the chloroethyl analogue of dacarbazine (DTIC), has significant activity against L1210 leukemia, as well as murine Sarcoma 180, adenocarcinoma 755, and Walker 256 tumors (1,2), and is more active than DTIC (3,4). Analogous fluoroethyltriazenes are also highly effective chemotherapeutic agents, although their usefulness is limited by toxicity (5). Similarly, the chloroethylnitrosoureas (ClEtNU) have activity against brain tumors, Hodgkin's disease, and lung and colorectal cancers (6–9). These agents inhibit DNA synthesis by alkylating the O-6 position of guanine (10,11).

This demonstrated activity of chloroethylating agents led to the synthesis of a series of 2-chloroethyl sulfonates (12,13) with the rationale that these compounds might function as haloethylating agents. Of those synthesized, clomesone [2-chloroethyl (methylsulfonyl)methane sulfonate] is highly effective against the murine P388 leukemia (13) and has activity comparable to the ClEtNU in the National Cancer Institute screening program. Clomesone, like the ClEtNU, has been reported to form interstrand cross-links with cellular DNA (14) but is more selectively reactive in that it chloroethylates, but does not hydroxyethylate, purine bases in DNA (15). In addition, clomesone lacks carbamoylating activity (15), a factor that has been implicated in the production of toxicity by the ClEtNU (16,17). These properties of clomesone have prompted its development for clinical trials.

The present study describes the disposition of clomesone in mice following iv administration. A preliminary report of this work has been presented (18).

MATERIALS AND METHODS

Clomesone was initially synthesized at Southern Research Institute (13). [Ethyl-^{14}C]Clomesone (9.6 mCi/mmol) was obtained from the Drug Development Branch, National Cancer Institute. Radiochemical purity of the [^{14}C]clomesone, as determined by high-pressure liquid chromatography (HPLC), was 96%.

The efficiency of recovery of [^{14}C]clomesone from mouse plasma was determined in vitro by adding [^{14}C]-clomesone to plasma at final concentrations of 1, 10, 25, 50, and 100 μg/ml. After mixing, samples were ex-

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tracted with 3 volumes of acetonitrile and the resultant extracts were radioassayed. Under these conditions, the recovery of [^{14}C]clomesone was > 90% over the concentration range tested.

[^{14}C]Clomesone solutions for injection were prepared in 0.9% sodium chloride, adjusted to pH 8 with sodium hydroxide. The solutions contained clomesone at a concentration of 3.5 mg/ml and had specific activities of 5 μCi/mg. When these were maintained at 5°C, < 3% radiochemical degradation of clomesone occurred during a 6-hour period.

Female B6C3F$_1$ mice (Simonsen Laboratories, Inc, Gilroy, CA), weighing 18–20 g, were administered 35 mg/kg of [^{14}C]clomesone, iv, through a tail vein. At selected times after dosing, mice were anesthesized with diethyl ether and a single, terminal blood sample was collected from the axillary region of each animal into a heparin-coated tube. After chilling in an ice bath for approximately 5 minutes, blood samples were centrifuged to obtain plasma. Liver, kidneys, spleen, lung, muscle (gastrocnemius), fat, brain, and small intestines were collected separately and frozen on dry ice. In separate experiments, urine was collected over ice from mice maintained in glass metabolism cages.

Upon collection, a portion of each plasma sample was mixed with 3 volumes of acetonitrile and centrifuged. The acetonitrile extract, which contained noncovalently bound clomesone and metabolites, was assayed for radioactivity and also analyzed by HPLC as described below. The precipitated plasma proteins (pellet) were radioassayed after solubilization in Soluene 350 tissue solubilizer (Packard Instrument Company, Inc, Downers Grove, IL). In addition, the remainder of each whole plasma sample was assayed for radioactivity. Urine samples were radioassayed without prior treatment and also analyzed by HPLC after mixing with 1 volume of acetonitrile. Tissues were weighed and then homogenized, without prior thawing, in 3 volumes of acetonitrile using a Polytron homogenizer equipped with a P-10 generating head (Brinkmann Instruments, Westbury, NY). Portions of each homogenate were digested with Soluene 350 tissue solubilizer and subsequently radioassayed. Another portion of tissue homogenate was centrifuged. The acetonitrile layer (extract) was removed and radioassayed; for liver samples, this extract was also assayed by HPLC as described above. To determine the extent of binding of radiolabel to trichloroacetic acid (TCA)-precipitable macromolecules, the tissue pellet was washed three times with 5% TCA and once with diethyl ether. The washed precipitates were dissolved in Soluene 350 and radioassayed.

For aqueous radioactive samples, the scintillator used was Safety Solve counting cocktail (Research Products International Corp, Mount Prospect, IL). Liquiflor (New England Nuclear, Boston, MA) was added to those samples digested in Soluene 350. All radioanalyses were accomplished in a liquid scintillation spectrometer equipped with an external standard.

HPLC analyses were accomplished with either a Waters Associates (Milford, MA) series ALC 244 HPLC equipped with a Packard Instruments TRI-CARB RAM 7500 or a Waters series 204 instrument equipped with a model R401 differential refractive index detector. With the exception of urine samples, resolution of sample components was achieved isocratically with a Partisil ODS-2 column (Whatman, Hillsboro, OR) and a mobile phase of acetonitrile:water (30:70) at 1 ml/min. One-milliliter fractions of the eluting material were collected and radioassayed after the addition of Safety Solve counting cocktail. Under these conditions of analysis, clomesone had a retention time of 12 minutes. For the analysis of urine, gradient elutions were accomplished at 1 ml/min with Waters Program 6 (0% solvent B, initial, to 100% solvent B, final, in 20 mins) with 25 mM $NH_4H_2PO_4$, pH 3.5 (solvent A), and acetonitrile:25 mM $NH_4H_2PO_4$, pH 3.5 (30:70) (solvent B). The limit of detection of [^{14}C]clomesone, which was fixed by the specific activity of the labeled compound, was about 2 ng. Essentially 100% recovery of radiolabel was obtained for all injected samples.

Binding of clomesone to plasma proteins was determined in vitro using Centriflo ultrafiltration membrane cones, type CF 25 (Amicon Corp, Danvers, MA). Mixtures of mouse plasma or water containing 10, 50, or 100 μg/ml of [^{14}C]clomesone were added to individual cones within 10 minutes of preparation and the cones were centrifuged at 5°C. The extent of [^{14}C]clomesone binding to plasma proteins was calculated from the difference between the radioactivity in the initial mixtures and that in the filtrates.

Pharmacokinetic half-lives were estimated using a modified form of NONLIN (19) and CSTRIP (20). The data were fitted to one-, two-, and three-compartment open models. A model was accepted as the best fit if an additional term, or compartment, failed to reduce significantly ($P < 0.05$) the weighted sums of squared errors as estimated by F test with appropriate degrees of freedom. Statistical weights were determined from the measured concentrations and were the same for each model.

RESULTS

In Vitro Experiments

When incubated in mouse plasma at 37°C, [^{14}C]clomesone was rapidly degraded with a half-life of < 2 hours (fig 1). This degradation was associated with progressive increases in both the percentage of acetonitrile-soluble metabolites formed (data not shown) and in the amount of clomesone-derived radioactivity that remained associated with plasma protein components. At 5°C, a substantially slower rate of clomesone disappearance occurred. At this temperature, acetonitrile-soluble metabolites were not formed but the amount of radioactivity that became associated with plasma proteins increased with time. In frozen plasma, [^{14}C]clomesone was stable for at least 2 days. In water at room temperature, the half-life of clomesone was about 24 hours.

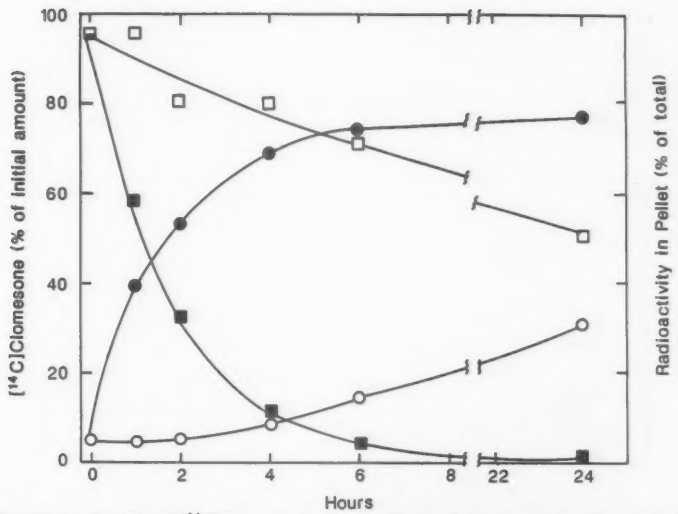

FIGURE 1.—Stability of [^{14}C]clomesone in plasma. Mouse plasma containing 25 μg/ml of [^{14}C]clomesone was incubated at 5°C (open symbols) or 37°C (closed symbols). At the indicated times, portions of each mixture were precipitated with 4 vols of acetonitrile. The resultant extracts were assayed for [^{14}C]clomesone (□, ■) by HPLC. The residual pellets (○, ●) were radioassayed after solubilization in Soluene 350. Results are the average of 2 determinations.

As determined by ultrafiltration at 5°C, about 50% of [^{14}C]clomesone was bound to mouse plasma proteins.

[^{14}C]Clomesone Disposition in Mice

Following iv administration of 35 mg/kg of [^{14}C]-clomesone to mice, clomesone was eliminated from plasma in an apparent single phase with a half-life of 11 minutes (fig 2A). Total radioactivity was eliminated from plasma with a half-life of 51 minutes during its initial phase of disappearance. Although the terminal phase of elimination of radioactivity could not be reliably estimated, it was of such a rate that plasma levels of radioactivity 24 hours after dosing were > 10 μg equivalents/ml. In addition to [^{14}C]clomesone, at least two radiolabeled metabolites, which eluted immediately after the solvent front during HPLC analysis, were present in the acetonitrile extracts of plasma. Although their identity was not directly investigated, one component eluted with a retention time identical to that of 2-chloroethanol, a clomesone degradation product formed in vitro (Y. F. Shealy, unpublished results). Plasma levels of these two polar metabolites increased during the first 60 minutes after dosing and then declined at a slower rate than that observed for clomesone (fig 2B). With time after dosing, the proportion of radioactivity in plasma that was associated with plasma protein components progressively increased such that, at 6 hours, essentially all of the remaining radioactivity was found in the acetonitrile precipitates of plasma (fig 2B).

Of the tissues collected, highest concentrations of radioactivity were in liver, kidney, and small intestines where, at most times of analysis, the levels were two to three times higher than in plasma (fig 3A). For these tissues, radioactivity levels progressively increased during the first 30 minutes after [^{14}C]clomesone administration and then declined at a rate slower than in plasma. In lungs, spleen, brain, and muscle, peak levels of radioactivity were reached within 5 minutes after dosing. Beyond this time, radioactivity was eliminated from these tissues at a rate similar to the rate in plasma. Little radioactivity was detected in fat at any time (data not shown). With few exceptions, $< 25\%$ of the total radioactivity in tissues was extractable into acetonitrile, indicating that the levels of unbound clomesone and organic soluble metabolites were low. Tissues that had the highest levels of radioactivity (eg, liver, kidney) had the lowest percentages of acetonitrile-extractable radioactivity and, conversely, tissues that contained the lowest levels of radioactivity (eg, lungs, muscle, and spleen) had the highest percentages of acetonitrile-extractable radioactivity (fig 3B). Only acetonitrile extracts of liver were analyzed for clomesone concentrations. At 5, 15, 30, and 60 minutes after dosing, the levels of unbound clomesone in liver were 2.1, 0.4, 0.2, and < 0.1 μg/g, respectively; clomesone was not detectable at later times. These concentrations were 15–50-fold lower than those in plasma at the same times. Following successive washing of tissue homogenates with TCA, a large proportion of the tissue radioactivity remained associated with macromolecular components (fig 3C). The highest levels

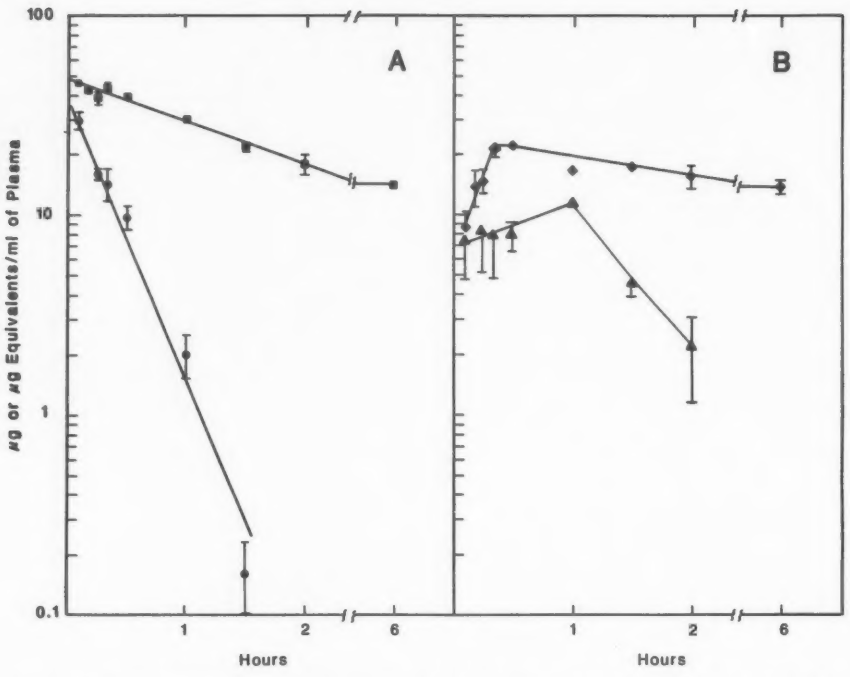

FIGURE 2.—Plasma levels of clomesone and clomesone-derived radioactivity after iv administration of 35 mg/kg of [^{14}C]clomesone to mice. (A) ● = clomesone; ■ = total radioactivity. (B) ▲ = acetonitrile-extractable metabolites; ◆ = protein-associated radioactivity. Values represent the mean ± SD for at least 3 mice at each time of analysis. In some instances, the SD was too small to be presented on the figure.

of TCA-precipitable radioactivity were in liver and kidney.

In four mice, 60.3% (SD ± 9.8) of the administered dose was excreted into urine within 8 hours. Of the radioactivity recovered, 2.9% (< 2% of the dose) was unchanged [^{14}C]clomesone. The total urinary recovery for 24 hours was 77.6 ± 5.2% (SD) of the dose. HPLC analysis of 8-hour urine collections established the presence of at least four radiolabeled metabolites of clomesone. These products comprised 13.9%, 45.3%, 22.7%, and 15.3%, respectively, of the recovered radiolabel. Their identity was not determined.

DISCUSSION

These studies demonstrated that clomesone is a reactive compound which, in mice, is rapidly and extensively distributed and metabolized. Following iv administration of clomesone, the half-life (11 mins) for disappearance from plasma was intermediate to the reported half-lives of the related chloroethylnitrosoureas carmustine (BCNU) (15 mins) (21) and lomustine (CCNU) (5 mins) (22) and the alkylating agents thiotepa (10 mins) (23) and melphalan (L-PAM) (24 mins) (24). In contrast to the rapid clearance of intact clomesone, total radioactivity was eliminated from plasma at a relatively slow rate. The difference between the initial rate of elimination of clomesone and that of total radioactivity was at least partially attributable to the progressive increase in the amount of clomesone-derived radioactivity that became associated with plasma protein components during the first 30 minutes after dosing. The prolonged second phase of disappearance of total radioactivity was presumably related not only to the binding of radioactivity to plasma proteins but also to the flux of radioactivity from tissues into plasma. A biphasic elimination of plasma radioactivity has been observed following administration of radiolabeled formulations of BCNU (25), PCNU [1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea] (26), and other alkylating agents (22,23,27) to various experimental animals.

In tissues, total radioactivity was highest in liver, kidneys, and small intestines, where the levels were twofold to threefold higher than in plasma at all times of analysis. These results indicated that the distribution of clomesone-derived radioactivity was qualitatively similar to the distribution of radiolabel following administration of BCNU, CCNU, and PCNU to mice (22,25,26). The low hepatic levels of organic-extractable clomesone were indicative of either a rapid metabolism or a high degree of binding of the compound by this

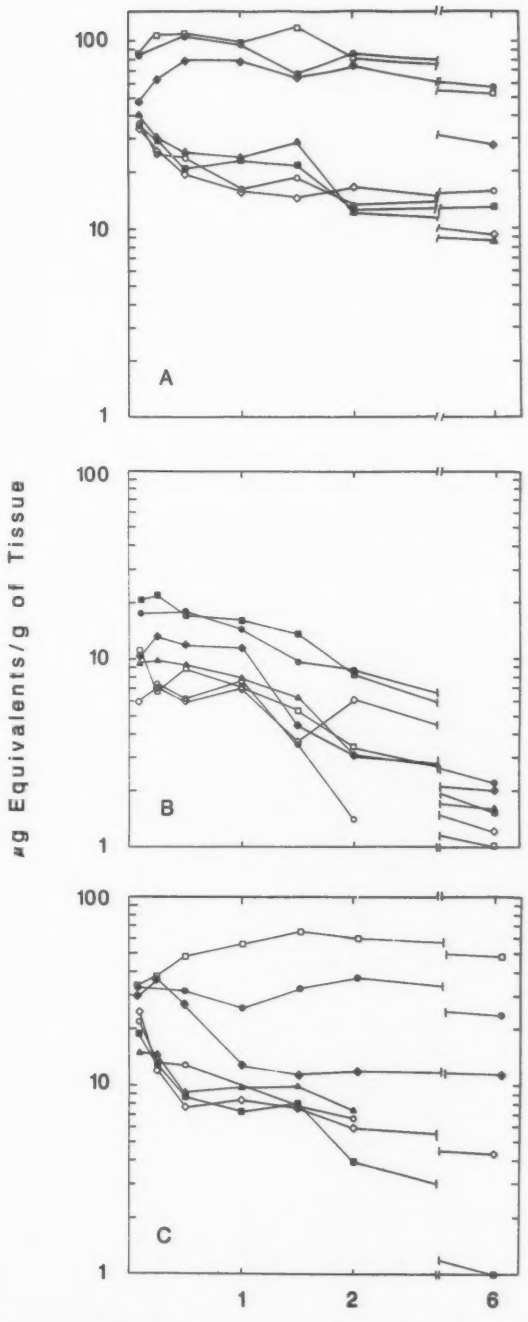

FIGURE 3.—Tissue levels of total radioactivity (A), acetonitrile-extractable radioactivity (B), and TCA-precipitable radioactivity (C) in mice after iv administration of 35 mg/kg of [^{14}C]clomesone. Tissues were obtained from the same mice presented in fig 2 and were pooled prior to analysis. □ = liver; ● = kidney; ○ = spleen; ▲ = lungs; ■ = brain; ◇ = muscle; and ◆ = small intestine.

tissue. With the exception of fat, levels of radioactivity in other tissues were essentially equivalent at all times after dosing and approximated the concurrent level in plasma. Analogous to the distribution of the lipophilic nitrosoureas (25,28) and other alkylating agents, such as L-PAM (29), significant levels of radioactivity were found in brain after [^{14}C]clomesone administration, indicative of the passage of the compound or its products into the central nervous system. The amount of radioactivity associated with TCA precipitates of tissues indicated that a large proportion of radioactivity was bound to macromolecular components. These results were consistent with the high reactivity of the compound and the reported intracellular binding of [^{14}C]-clomesone to DNA (14). Similar degrees of macromolecular binding have been reported for related alkylating agents (26,28).

Urinary excretion was a major route of elimination of radioactivity derived from [^{14}C]clomesone, in that > 75% of the dose was recovered in urine within 24 hours. Comparable degrees of urinary elimination have been reported following administration of either BCNU or CCNU to mice (22,25). HPLC analysis of urine indicated the presence of only low levels of intact clomesone (< 2% of the dose), results which confirmed the extensive metabolism and/or degradation of the compound. Although the identity of the four urinary metabolites was not investigated, some were possibly mercapturic acid derivatives. Similar compounds were present in the urine of rats dosed with RPCNU [1-(2-chloroethyl)-3-(2′,3′,4′-tri-*O*-acetylribopyranosyl)-1-nitrosourea] (27). Other possible metabolites were thiodiacetic acid and its sulfoxide derivative, components that have been identified as metabolites of chloroethylnitrosoureas (27,30).

In contrast to its rapid metabolism in vivo, clomesone displayed moderate stability in aqueous solution in vitro, with an apparent half-life of about 24 hours in water at 25°C. That clomesone is not rapidly degraded in vitro should promote its clinical usefulness.

The results of this study indicate that clomesone and/or its metabolic products share common features of distribution and tissue binding with related nitrosoureas. In view of its more selective reactivity, however, clomesone may not produce the same toxic effects exhibited by these agents.

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Semiautomated Colorimetric Assay for In Vitro Screening of Anticancer Compounds[1,2]

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An in vitro tetrazolium dye (MTT) reduction technique was modified and evaluated for use in the large-scale screening of anticancer compounds by examining the activity of ten clinically used drugs against 16 different human and murine cell populations. Cell populations included colon and mammary adenocarcinomas, melanomas, leukemias, and freshly isolated normal cells. Cell lines were grown in microtiter plates for 18–20 hours prior to a 72-hour continuous exposure to the drugs. Cultures were initiated at cell densities which maximized both the difference in dye reduction and the number of cell doublings between the beginning and end of the drug exposure period. Drug potency, expressed as the 50% inhibitory concentration (IC50), was comparable whether the effect on cell doublings or dye reduction was determined. There was good agreement between this method and the more labor-intensive, conventional method of counting trypan blue dye-excluding cells in a hemacytometer. Implemented as a large-scale, high-capacity system, our adaptation of the MTT technique is a rapid, sensitive, reproducible first-line screening device for detecting anticancer compounds with cytostatic or cytocidal activity. [Cancer Treat Rep 71:1141–1149, 1987]

One approach to the development of new anticancer drugs is based (in part) on the initial screening of chemical compounds in human or murine tumor cells in vitro. In this step, compounds can be identified, evaluated, and prioritized for more extensive testing using cell survival as an index of chemosensitivity. Techniques currently used to assess chemosensitivity include eosin Y, erythrocin B, nigrosin, or trypan blue dye exclusion with or without internal standards (1–8), neutral red dye uptake (9,10), differential staining of cell suspensions with fast green/nigrosin or fast green/hematoxylin/eosin (11,12), Giemsa or crystal violet staining of fixed cells (13–16), Hoechst 33342-DNA chromatin binding (17), cellular ^{14}C-CO_2 production from ^{14}C-labeled glucose (18,19), ^{3}H-thymidine or ^{3}H-uridine incorporation into nucleic acids (20–23), fluorescein dye hydrolysis (24,25), Coulter counts of single cell suspensions (23,26,27), and the soft agar clonogenic assay with or without image analysis (23,28–31).

Since the probability of discovering new drugs increases in proportion to the number of compounds tested, it follows that drug discovery programs are best served by assays which combine speed and high throughput capacity with sensitivity, reproducibility, and relevance to the desired target organ system. Each of the previously mentioned methods yields unique and valuable information. However, the vast majority have technical constraints, ranging from extensive processing time to poor reproducibility, which limit their usefulness for large-scale screening programs which are targeted to evaluate thousands of compounds each year.

The tetrazolium dye (MTT) assay first described by Mosmann (32) is a noteworthy alternative for use in such programs. Based on the selective ability of living, but not dead, cells to reduce the yellow tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) to a purple formazan, the number of viable cells is spectrophotometrically measurable as the concentration of the MTT reaction product. This type of assay has proven useful in measuring the effects of proliferative (32–34) and cytotoxic lymphokines (35), mitogen stimulation, and complement-mediated lysis (32), in assessing radiosensitivity (36), and in evaluating the chemosensitivity of L1210 murine leukemia cells (37) and human lung tumor cells (38,39). Some earlier biological applications of tetrazolium salts include detection of virus-induced cytopathology in vitro (40) and in vitro and in vivo metabolism of normal and malignant tissues (41–43).

In this report, we describe our adaptation of the MTT

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assay (44) and the subsequent development of a rapid, sensitive, reproducible method with the potential for screening several thousand compounds a year against a wide variety of cell populations.

MATERIALS AND METHODS

Cells

Human cells were as follows: colon adenocarcinomas COLO201 (ATCC CCL224), COLO205 (ATCC CCL222), COLO320HSR (ATCC CCL220.1), LoVo (ATCC CCL229), WiDr (ATCC CCL218), and DLD-1 Clone A (45); mammary adenocarcinoma MCF7 (ATCC HTB22); chronic myelogenous leukemia K562 (ATCC CCL243); amelanotic melanoma RPMI7272 (46), and freshly isolated normal peripheral blood mononuclear cells (HPBMC).

Murine cells were as follows: lymphocytic leukemia L1210 (ATCC CCL219); transformed fibroblasts L929 (ATCC CCL1); melanoma B16 and the high and low metastatic variants B16F10 and B16F1, respectively (47), and freshly isolated normal spleen cells.

With the exception of L1210, K562, and LoVo, all cell lines were maintained as subconfluent monolayers by twice-weekly passage in RPMI-1640 tissue culture medium with L-glutamine supplemented with 10% heat-inactivated fetal bovine serum (GIBCO Laboratories, Grand Island, NY), 25 μg/ml gentamicin sulfate (Tri-Bio, State College, PA), and 50 μM 2-mercaptoethanol (Sigma Chemical Co, St. Louis, MO) (RPMI-G). L1210 and K562 were maintained in RPMI-G as stationary suspension cultures. LoVo was maintained as a subconfluent monolayer in RPMI-G supplemented with 20% fetal bovine serum. All cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air.

For subculturing and initiation of the assay on Day 1, DLD-1 Clone A, LoVo, MCF7, RPMI7272, WiDr, L929, B16, B16F1, and B16F10 monolayers were dissociated to single-cell suspensions using 0.25% trypsin-EDTA. Due to a relatively longer lag time following seeding (48), LoVo monolayers were enzymatically dissociated and seeded on Day 0. COLO201, COLO205, and COLO-320HSR monolayers were dissociated by vigorous pipetting. K562 and L1210 cells were harvested directly from suspension cultures. Cell concentration and viability were determined by hemacytometer counts of cells excluding 0.1% trypan blue dye. Suspensions were adjusted to a predetermined cell concentration which maximized the difference in dye reduction between the beginning and end of a 96-hour interval.

HPBMCs were isolated from fresh whole blood by Ficoll centrifugation (49). The number of mononuclear cells was determined by Coulter counter or hemacytometer and the concentration adjusted to 4×10^6 cells/ml in RPMI-G.

Normal spleen cells were obtained from 9–13-week BALB/c mice killed by cervical dislocation. The spleens were gently dissociated in 15 ml of RPMI-G using a loose-fitting Potter-Elvejhem tissue grinder. The resulting cell suspension was washed twice in fresh RPMI-G and the number of cells determined and adjusted as above.

For initiation of the assay on Day 2, 50 μl of HPBMC or spleen cell suspension was added to 50 μl of 8-μg/ml phytohemagglutinin (PHA, Wellcome Research Laboratories, Beckenham, England) in Ca++- and Mg++-free phosphate-buffered saline (PBS).

Drugs

Dactinomycin, doxorubicin, bleomycin, cytarabine (ara-C), cisplatin, cycloheximide, 5-fluorouracil, mitomycin, methotrexate, and vincristine (Sigma) were first solubilized in dimethyl sulfoxide (DMSO, Fisher Scientific Co, King of Prussia, PA) and then diluted with an equal volume of sterile distilled, deionized water. Half-log dilutions from 10 to 0.0001 μg/ml were made in RPMI-G. Drug solutions were made fresh on the day of assay.

MTT Reduction Assay

Day 1.—A total of 100 μl of cell suspension was added to all but one well of a flat- or round-bottom 96-well microtiter plate (COSTAR, Cambridge, MA). The single well ("blank") received 100 μl of RPMI-G only, as a control for nonspecific dye reduction. The plates were incubated at 37°C for 18–20 hours in a humidified atmosphere of 5% CO_2 in air.

Day 2.—A total of 100 μl of the drug solutions was added to quadruplicate wells. For each plate containing cell lines, quadruplicate growth control wells and blanks received 100 μl of fresh RPMI-G. Triplicate solvent control wells received 0.2% DMSO in RPMI-G. For each plate containing HPBMCs or spleen cells, an additional set of triplicate growth control wells received RPMI-G plus cells without mitogen. All plates were incubated at 37°C for 72 hours.

Day 5.—A total of 100 μl of fluid was removed from each well prior to the addition of 50 μl/well of 1 mg/ml of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (Sigma)) in PBS. After 3½–4 hours of incubation at 37°C, 100 μl of 0.04 N HCl in isopropanol was added to each well and mixed thoroughly. Within 1 hour at room temperature the absorbance of the wells was read in a Flow MCC scanning-well spectrophotometer (Flow Laboratories, Inc, McLean, VA) at test and reference wavelengths of 570 and 630 nm, respectively. Absorbance was recorded in optical density (OD) units. Absorbance of the blank was subtracted from that of the remaining wells. Drug potency was expressed as the 50% inhibitory concentration (IC50); ie, the drug concentration which inhibited cell growth by 50% based on a plot of the log drug concentration versus percentage of control absorbance. Raw data manipulation and calculation of IC50s were performed via interface with an IBM PC-XT computer with customized software.

RESULTS

To assess the suitability of the tetrazolium salt reduction technique as a quantitative assay for cell growth inhibition, a series of four preliminary experiments was done. Results are shown primarily for DLD-1 Clone A cells, although each series of experiments was performed using several different cell populations.

The first set of experiments determined the relationship between cell number and the amount of MTT reduced to formazan. The results in figure 1 show that formazan absorbance (in OD units) at 24 and 96 hours post-seeding was proportional to the number of cells seeded and that the relationship was hyperbolic from 8000 to 32 cells/well. This relationship was apparent at up to and including 128,000 cells/well (experiments done using LoVo cells, data not shown). In the LoVo experiments, the signal-to-noise ratio was 14:1 (average OD for blanks, 0.004 [range, 0.001–0.010]; average OD for 32 cells, 0.056 above blank [range, 0.048–0.065]). At very high concentrations (different for different cell populations) absorbance showed a plateau.

The second set of experiments, based on figure 1, established the direct relationship between OD and optimal seeding concentration for each cell population. Replicate 96-well plates were seeded with half-log dilutions ranging from 8000 to 32 cells/well. One plate each was processed on Days 2 and 5 as described in the *Materials and Methods* section. Optimal seeding concentration was determined from the cell concentration which maximized OD differences between Days 2 and 5 while avoiding the plateau region for cell number versus OD. Figure 2 summarizes the calculated optimal seeding concentrations for each of the cell populations.

The third set of experiments determined that the optimal seeding concentration maximized the number of cell doublings. Replicate 96-well plates were seeded with 1000, 2000 (optimal concentration, and 4000 DLD-1 Clone A cells/well. One pair of plates, from which the maximum change in OD was determined, was processed using the MTT technique. The second pair of plates, from which cell doublings were determined, was processed by trypsinization, pooling of replicate wells, and counting of trypan blue dye-excluding cells using a hemacytometer. This was done on Days 2 and 5 for replicate plates. For each initial seeding concentration the number of cell doublings in 72 hours was calculated from the difference in cell numbers between Days 2 and 5. Tables 1A and 1B show that the optimal seeding concentration maximized not only the change in OD (table 1A) but also the number of cell doublings (table 1B).

The fourth set of experiments confirmed that IC50s based on 50% reduction in OD were comparable to those based on 50% reduction of cell doublings. Replicate 96-well plates were seeded with 2000 DLD-1 Clone A cells/well on Day 1. On Day 2 the cells of one plate were trypsinized and counted manually as previously described. This established a baseline for cell growth and doubling determination. The remaining plates received eight half-log dilutions of doxorubicin, ara-C, or mitomycin, two plates per drug. On Day 5 one plate per drug was evaluated using MTT and the other was evaluated by trypsinization and manual counts. An IC50 in the MTT assay was defined as the drug concentration required to decrease control absorbance by 50%. A trypan blue IC50 was defined as the drug concentration required to inhibit the number of control cell doublings

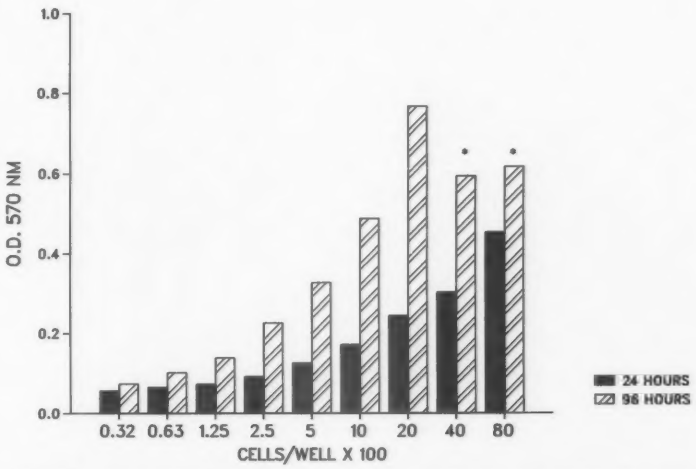

FIGURE 1.—OD vs cell number for DLD-1 Clone A cells. Absorbance of MTT formazan (in OD units) was related hyperbolically to cell number at 24 and 96 hrs after seeding of DLD-1 Clone A cells. At high cell concentrations absorbance shows a plateau possibly due to decreased metabolic activity in overcrowded wells (indicated by *).

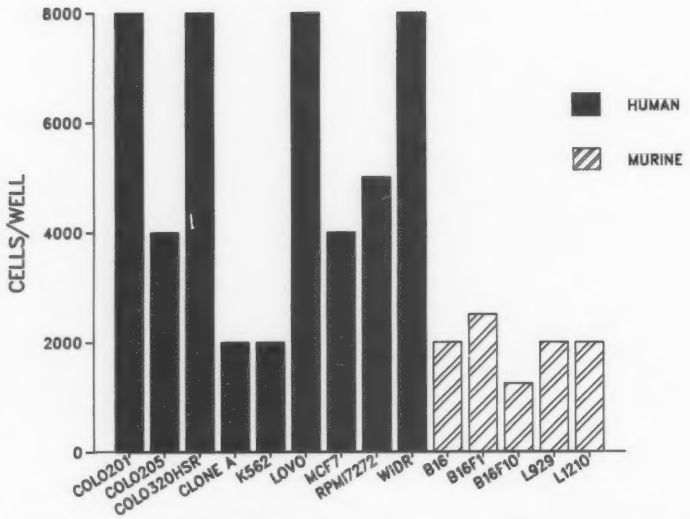

FIGURE 2.—Optimal seeding concentrations for cells used in the MTT dye reduction assay. Optimal seeding concentrations for the cell lines used in this study were determined by calculation of the maximum differences in OD between Days 2 and 5 using replicate 96-well plates. Human peripheral blood mononuclear cells and murine spleen cells were seeded at 2×10^5 cells/well.

by 50%. The results in table 2 show that IC50s determined by the two different methods were comparable ($P > 0.05$) and were different by at most twofold. The number of doublings in replicate plates of DLD-1 Clone A cells was within the limits of counting error for the trypan blue method (4.89 and 5.07 doublings for plates containing doxorubicin and mitomycin, respectively).

The dye reduction assay was reproducible over time and among different experimenters. Tables 3A and 3B show a summary of results obtained over a 7-month period for two human (DLD-1 Clone A and RPMI7272) and two murine (L1210 and B16) cell lines. Some variation in cell growth/dye reduction was noted in each cell line (table 3A: coefficient of variation, range 197%–354%). Interestingly, less variation was noted for drug IC50s in these same cells (table 3B: coefficient of variation, range 171%–180%). Cells were seeded at optimal concentration (fig 2) on Day 1 and received 100 μl/well of either fresh RPMI-G (growth controls) or graded concentrations of doxorubicin on Day 2 (either eight half-log dilutions or four one-log dilutions). Plates were processed on Day 5 as outlined in the *Materials and Methods* section.

Having established the suitability of the MTT technique for our purposes, this system was used to evaluate the growth-inhibitory effects of ten clinically used anticancer drugs on 16 different normal and transformed cell populations (ten human and six murine). Table 4

TABLE 1A.—Effect of optimal seeding concentration on OD in DLD-1 Clone A cells*

Experiment	Cells/well $\times 10^3$	OD		Change in OD
		Day 2	Day 5	
1	1	0.053	0.392	0.339
	2†	0.099	0.491	0.392
	4	0.193	0.578	0.385
2	1	0.030	0.371	0.341
	2†	0.081	0.491	0.410
	4	0.180	0.422	0.242

*Replicate 96-well plates were seeded with DLD-1 Clone A cells on Day 1. One pair of plates was processed by the MTT technique and the change in OD calculated from the OD differences on Days 2 and 5.

†Optimal seeding concentration.

TABLE 1B.—Effect of optimal seeding concentration on cell doublings in DLD-1 Clone A cells*

Experiment	Cells/well $\times 10^3$	Viable cells/ml		Cell doublings
		Day 2	Day 5	
1	1	6.3×10^2	1.7×10^4	4.6
	2†	1.2×10^3	3.2×10^4	4.7
	4	2.9×10^3	5.7×10^4	4.3
2	1	5.3×10^2	1.1×10^4	4.3
	2†	1.0×10^3	2.7×10^4	4.6
	4	1.9×10^3	2.4×10^4	3.6

*The cells in the second pair of plates were trypsinized and counted using a hemacytometer. Cell doublings were calculated from numbers of viable cells on Days 2 and 5 as described in the *Materials and Methods* section. Data shown are from 2 separate experiments, 32 wells/cell concentration.

†Optimal seeding concentration.

TABLE 2.—Comparison of IC50s (μg/ml) obtained using MTT dye reduction vs trypan blue dye exclusion*

Cell line	Doxorubicin		Ara-C		Mitomycin	
	MTT	Trypan blue	MTT	Trypan blue	MTT	Trypan blue
Clone A	0.050	0.060	—	—	0.06	0.05
B16	0.002	0.003	—	—	—	—
L1210	0.020	0.040	0.001	0.002	—	—

*There was good agreement between the MTT dye reduction technique and the more labor-intensive trypan blue dye exclusion method ($P > 0.05$). In vitro potency (IC50) based on 50% reduction in OD is comparable to that based on 50% inhibition of cell doublings (ie, within twofold variation). Replicate 96-well plates were seeded with DLD-1 Clone A, B16, or L1210 cells at optimal concentration on Day 1. The cells of one plate were trypsinized and counted on Day 2 using a hemacytometer to establish a baseline for cell growth. The remaining plates received graded concentrations of drugs. On Day 5, 1 plate/drug was evaluated using the MTT technique and the other by hemacytometer counts of pooled trypsinized cells.

summarizes this information and lists cell populations in order of decreasing overall chemosensitivity; ie, by decreasing average IC50. Examples of dose-response curves are shown in figure 3. Variation in replicate experiments was comparable with that previously indicated in table 3B.

DISCUSSION

An in vitro colorimetric dye reduction technique described by Mosmann (32) was modified and evaluated for use in the large-scale screening of anticancer compounds. The assay is based on the selective reduction of the tetrazolium salt MTT by cells which remain viable after a 72-hour exposure to anticancer drugs. Mitochondrial dehydrogenases at the sites of cytochromes *b* and *c* (50–52) convert the yellow form of the salt to an insoluble, intracellular purple formazan. Formazan is then solubilized with isopropanol to a homogeneous solution for spectrophotometric measurement of absorbance/OD at test and reference wavelengths of 570 and 630 nm, respectively. Addition of 0.04 N HCl to the alcohol acidifies the phenol red indicator in the RPMI-G to its fully acidic yellow form to minimize absorption interference at the test wavelength (32).

Sixteen different normal and transformed cell populations were used in this study: ten human and six murine. Generation of MTT formazan, as reflected in OD, was related hyperbolically to the number of viable cells present 24 and 96 hours postseeding. This relationship was unaffected by the presence of increasing numbers of nonviable cells (32,35, and R. L. Ruben, unpublished results) and was generally maintained at cell concentrations ranging from 32 to 128,000 cells/well, depending on the cell population. A similar relationship between OD and cell number was also reported by Mosmann (32), Denizot and Lang (34), and Green et al (35) for nonadherent cells and by Cole (38) for adherent cells. At high cell concentrations (which varied among the cell populations), absorbance showed a plateau possibly due to decreased metabolic activity in overcrowded wells. This was also noted by Denizot and Lang (34) and by Green et al (35). Because of the hyperbolic nature of the OD-cell number curve, the need for proper calibration and the importance of avoiding cell concentrations in the plateau region of the curve cannot be overemphasized.

We found the dye reduction assay to be sensitive enough to detect as few as 32 viable cells/well with a signal-to-noise ratio of 14:1. Denizot and Lang (34) modi-

TABLE 3A.—Reproducibility of cell growth based on OD in the MTT dye reduction assay*

Cell line	No. of tests	OD ± 95% confidence limit
Clone A	232	0.370 ± 0.169
RPMI7272	92	0.483 ± 0.194
L1210	175	0.580 ± 0.235
B16	88	0.377 ± 0.159

*Reproducibility of the dye reduction assay was determined among various experimenters over a 7-mo period. Cells were seeded at optimal concentration (fig 2) on Day 1. On Day 2, 100 μl/well of fresh RPMI-G was added to quadruplicate wells. Plates were processed by the MTT technique on Day 5.

TABLE 3B.—Reproducibility of drug potency based on the IC50 of doxorubicin in the MTT dye reduction assay*

Doxorubicin vs—	No. of tests	IC50 (μg/ml) Mean ± 95% confidence limit
Clone A	100	0.050 ± 0.0176
RPMI7272	92	0.008 ± 0.0029
L1210	78	0.020 ± 0.0078
B16	73	0.002 ± 0.0007

*Reproducibility of the dye reduction assay was determined among various experimenters over a 7-mo period. Cells were seeded at optimal concentration (fig 2) on Day 1. On Day 2, 100 μl/well of graded concentrations of doxorubicin was added to quadruplicate wells. Plates were processed by the MTT technique on Day 5.

TABLE 4.—Chemosensitivity of cells based on IC50s (μg/ml) of some clinical anticancer drugs in the MTT dye reduction assay*

Cells	Dactino-mycin	Doxorubicin	Ara-C	Bleomycin	Cisplatin	Cyclo-heximide	5-FU	Mitomycin	Metho-trexate	Vincristine	Average IC50
B16F10	< 0.0001	0.001	0.006	0.01	0.001	0.04	0.01	0.008	< 0.0001	< 0.001	0.008
B16	< 0.0001	0.002	0.005	0.05	0.06	< 0.001	0.01	0.002	< 0.0001	0.001	0.013
B16F1	< 0.0001	0.001	0.0002	0.11	0.35	< 0.001	0.002	0.01	< 0.0001	< 0.001	0.048
L929	< 0.0001	0.1	0.46	0.04	0.30	0.05	0.29	0.08	< 0.0001	0.009	0.132
Mouse spleen	< 0.001	0.05	0.07	0.32	> 1	0.03	0.02	0.03	0.003	0.004	0.153
HPBMC	< 0.001	0.02	0.04	> 1	> 1	—	0.04	0.03	0.007	2.30	0.493
LoVo	< 0.001	0.02	0.10	0.07	> 10	0.02	0.44	0.04	0.45	0.002	1.114
K562	< 0.0001	0.003	0.01	0.10	> 10	0.01	0.02	0.03	1.56	0.01	1.174
RPMI7272	< 0.0001	0.008	0.44	3.60	> 10	0.02	0.99	0.02	0.0003	< 0.001	1.508
COLO320HSR	< 0.001	0.17	0.03	7.90	9.50	0.03	0.44	0.19	0.46	0.03	1.875
L1210	< 0.001	0.02	0.001	> 10	> 10	0.01	0.004	0.006	< 0.001	0.007	2.005
Clone A	< 0.0001	0.05	0.14	> 10	> 10	0.10	0.21	0.06	> 10	0.01	3.057
WiDr	< 0.0001	0.17	> 10	> 10	> 10	0.13	1.50	0.46	> 10	< 0.001	4.226
COLO205	< 0.001	0.35	> 10	> 10	> 10	0.03	> 10	0.11	3.70	< 0.001	4.419
MCF7	< 0.0001	0.01	> 10	> 10	> 10	0.53	0.49	0.04	> 10	10	5.107
COLO201	< 0.001	> 1	> 10	> 10	> 10	0.31	> 10	9.20	> 10	> 10	7.051

*In vitro potencies of 10 clinically used anticancer drugs were determined in 16 different cell populations using the MTT technique as described in the *Materials and Methods* section. Cells are listed in order of decreasing overall chemosensitivity. Each IC50 represents the mean of at least 4 independent tests, each run in quadruplicate. Several entries are listed as > or < values. To us, these IC50s sufficiently indicated sensitivity (< 0.0001 μg/ml) or resistance (> 10 μg/ml) relative to other cells and/or drugs without the need to retest at higher or lower concentrations. Decreasing overall chemosensitivity of the cell populations was reflected in decreasing average IC50s. In ranking cell populations according to average IC50, the numerical value alone was used in cases where the IC50 was expressed as > or <.

fied the Mosmann assay to improve sensitivity and reliability in measuring the effect of proliferative lymphokines and reported a minimum detectable cell number of 1000.

Formazan was completely solubilized with the acid isopropanol, and OD readings remained stable over a 2-hour period regardless of the number of viable cells generating the signal. This is in contrast to reports from other laboratories indicating that acid isopropanol was not a suitable solvent for MTT formazan. Alley et al (53)

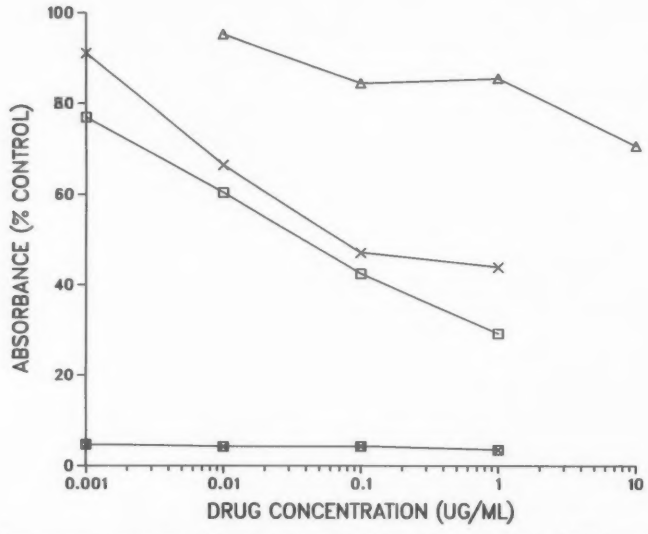

FIGURE 3.—Dose-response curves for 4 drugs tested against DLD-1 clone A cells in the MTT dye reduction assay. Decreasing numbers of viable cells (measured as decreasing absorbance with respect to controls) were generally associated with increasing doses of drugs. IC50s in the test shown are as follows: cisplatin, > 10 μg/ml; ara-C, 0.16 μg/ml; doxorubicin, 0.04 μg/ml; and dactinomycin, < 0.001 μg/ml. △ = cisplatin; X = ara-C; □ = doxorubicin; and ⊠ = dactinomycin.

reported signal fading in some cell lines and overcame the problem by replacing acid isopropanol with 100% DMSO. Denizot and Lang (34) used pure ethanol or propanol and read OD at test and reference wavelengths of 560 and 690 nm, respectively. Carmichael et al (36,39) replaced acid isopropanol with DMSO in suspension cultures and with mineral oil in monolayer cultures and read the results at test wavelengths of 540 and 570 nm, respectively.

Optimal Day 1 seeding concentrations were determined for each cell line by calculating the maximum difference in OD between Days 2 and 5 using replicate 96-well plates. As expected, this concentration varied among cell lines (reflecting differences in cell doubling times [R. L. Ruben, unpublished data]) but was generally between 2×10^3 and 8×10^3 cells/well. Optimal seeding concentration maximized the number of cell doublings over the 72-hour drug exposure period. Although normal cells were seeded at 2×10^5 cells/well, only a small subpopulation was capable of PHA-stimulated proliferation over 72 hours. Therefore, the number of actively dividing cells was not substantially different from that of the tumor cell lines.

In vitro drug potencies (IC50s) based on 50% inhibition of cell doublings were comparable to those based on 50% reduction in OD. The former had been used routinely in IC50 calculations in our laboratory in conjunction with a cell growth inhibition assay in which viable cells from suspension cultures or trypsinized monolayers were counted using a hemacytometer. It was noted that some MTT and trypan blue IC50s were nearly identical and others differed by up to twofold. Thus, direct comparison of data obtained by either method could be made without conversion factors.

There was good agreement between the dye reduction assay and the more conventional trypan blue dye exclusion method. Cell morphology and relative density of the cultures (cell growth) were equivalent for the corresponding control and drug-treated cells. Results obtained using other MTT-based assays correlated well with the microculture cellular protein assay (53), ^{3}H-thymidine uptake (33), Coulter counter analysis (37), the clonogenic assay (36,39), and the dye exclusion method of Weisenthal et al (11) as reported by Carmichael et al (36,39).

The dye reduction assay was reproducible, as assessed by relative consistency of control cell ODs and drug IC50s among various experimenters over a 7-month period. Some variation in cell growth/dye reduction was noted in each cell line. This could be due to the effects of continuous passage in culture or due to technical differences in cell handling or in performing the assay. Less variation was noted for drug IC50s. Thus, despite variations in growth over time, the MTT assay, like other bioassays, can provide reasonably precise estimates of drug potency. Minor changes in culture media, incubator conditions, method of drug dilution, and MTT dye lot did not affect OD of the control cells or IC50s of the test drugs (R. L. Ruben, unpublished data). Acceptable cell growth ($\geq$ three cell doublings) could be attained over a twofold range of seeding densities (eg, 1×10^3–2×10^3 DLD-1 Clone A cells/well).

IC50s of ten clinically used anticancer drugs were determined in 16 different human and murine cell populations which included colon and mammary adenocarcinomas, lymphocytic and chronic myelogenous leukemias, melanoma, fibroblasts, and normal spleen and peripheral blood mononuclear cells. Among the different classes of drugs tested were alkylators, antibiotics, antimetabolites, and vinca alkaloids. Results indicated a range of chemosensitivity: cells in which IC50s were > 10 μg/ml were more resistant; those < 0.001 μg/ml were more sensitive. Murine cells were generally more sensitive to the drugs than were human cells, but sensitivity differences within a species were also apparent. These intraspecies differences may be due to such factors as tissue of origin or growth rate. Such inherent differences in chemosensitivity could be exploited in large-scale screen designs using cell populations with high, moderate, and low levels of sensitivity to marketed anticancer agents. Differences in sensitivity between tumor cells and normal cells were not observed.

An IC50 was used as the index of drug potency rather than an IC90, for example, for several reasons. First, the IC50 lies on the linear portion of the classic sigmoidal drug dose-response curve of log drug concentration versus response. Therefore, the IC50 allows a reasonable estimation of drug potency with a limited number of drug concentrations. Second, an IC90 imposes a rather stringent criterion for potency of inhibition in a first-line screen, which is one of the major functions of the MTT system in our laboratory. Finally, it has been our experience that an IC50 provides reproducible results.

The assay was designed to yield drug potency data after a 72-hour drug exposure (assay Day 5). This experimental design allows a balance between sufficient amount of cell growth and a high through-put capacity for evaluating large numbers of compounds. Because this is a relatively short-term assay, it is possible that some agents which induce substantially delayed toxicity may not be detected, a phenomenon noted by Weisenthal et al (8). For many agents, however, delayed or cumulative toxicity is usually apparent after two to three cell doublings. These compounds would not be missed in our system, in which cells typically undergo a minimum of four doublings.

The MTT dye reduction assay as described in this report has several important advantages over many of the conventional systems mentioned earlier. It is conceptually and mechanically simple. Since radioactivity is not involved, no radiation training, precautions, and monitoring are required (training and monitoring are required, however, to protect workers from chemical and biological hazards). The assay is sensitive even at low cell numbers and is reproducible over time and among different experimenters. A standard tissue culture laboratory requires no additional equipment except for a benchtop scanning-well spectrophotometer; com-

puter interface is optional but recommended. The few reagents required are inexpensive, used in very small quantities, and stable for relatively long periods: MTT in solution can be stored protected from light at 4°C for several months; MTT powder is stable for at least 2 years in a dessicator at 4°C; and the acid alcohol is stable indefinitely at room temperature. The OD signal that is generated is stable for up to 2 hours at room temperature, and the technique is applicable to a wide variety of cell types. It does not require subsets of specifically adapted cells and uses intact cells in which two-dimensional (monolayers) and three-dimensional (suspensions) intercellular interactions are preserved. Since MTT is nontoxic, drug-exposed cells can be recovered (54) prior to the addition of alcohol. Compounds can be evaluated at multiple concentrations either singly or in combination simultaneously on a number of different cell lines. The assay detects cytostatic as well as cytocidal compounds and, since it requires no washing, cell dissociation, or manual counting, it is rapid and readily lends itself to automation, thereby reducing the potential for human error.

We note two limitations to the MTT assay. First, as the test is described, it cannot distinguish between cytostatic and cytocidal compounds; ie, those associated with drug-induced delay in cell division versus reproductive cell death. This limitation was also noted by Carmichael et al (36). To make the distinction between cytostatic and cytocidal effects, the growth potential of drug-treated cells would have to be determined subsequently in the absence of drug. This limitation can be overcome by a change in experimental design. Second, in the MTT assay individual cell numbers are not quantitated. Instead, drug effect on an entire cell population is reflected as a percentage of control absorbance. This limitation, unlike the first, is inherent in the use of this dye. Other investigators report light scatter interference caused by an opaque alcohol-induced protein precipitate which forms in the presence of serum concentrations of 10% (34) or > 15% (35). The precipitate can be solubilized by adding 20 μl/well of 3% wt/vol sodium dodecyl sulfate in water (35) or can be avoided by using serum-free medium for the incubation step with MTT (34). We have not found protein precipitation to be a frequently occurring problem.

Other assays which are adaptable to large-scale screening of anticancer compounds include neutral red dye uptake by lysosomes of viable cells (9,10), production of ^{14}C-CO_2 by cells grown in ^{14}C-glucose-containing medium [the Bactec system (18,19)], and Hoechst 33342 fluorescence of DNA chromatin (17). We have not pursued these assays because of our desire to avoid (*a*) radioactivity (Bactec system), (*b*) washing steps which could increase processing time and sample variability (neutral red, Hoechst), and (*c*) the need to alter the number of steps or the basic parameters of the test to accommodate different cell lines (Hoechst).

In summary, our adaptation of the MTT dye reduction technique provides a rapid, sensitive, reproducible means of quantitating anticancer drug potency in vitro against a wide variety of cell types. Implemented as a large-scale, high-capacity system, it has the potential for being a valuable first-line screening device for detecting new anticancer compounds with cytostatic or cytocidal activity.

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Oral Versus Im Administration of High-Dose Medroxyprogesterone Acetate in Pretreated Patients With Advanced Breast Cancer[1,2]

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In a multicenter trial, 123 patients with advanced breast cancer who had been treated with tamoxifen and/or chemotherapy were randomized to receive medroxyprogesterone acetate (MPA) orally 300 mg × 3 daily or im 500 mg daily for 4 weeks and 500 mg × 2 weekly thereafter. All case histories were reviewed extramurally by the criteria of the International Union Against Cancer. Five and 11 patients were not eligible and evaluable for response, respectively. Pretreatment characteristics were well balanced in both treated groups. Twenty-five of all 107 (23%) evaluable patients achieved an objective remission, whereas in a further 15% the disease became stable after previous progression. Results in both treatment arms did not differ significantly. The median duration of objective remission was 12 and 14 months for orally and im treated patients, respectively ($P > 0.10$). No statistically significant differences in the survival times of orally and im treated patients were found. Pretreatment characteristics positively correlated with an objective remission during MPA therapy in both groups were age > 50 years ($P < 0.02$) and no previous chemotherapy ($P < 0.01$). Toxicity included an increase in body weight, cushingoid effects, muscle cramps, and tremors in both groups. In four patients on im therapy, local infections developed. Mean serum MPA levels reached values above 100 ng/ml in nine orally and eight im treated patients ($P > 0.10$), and neither differed significantly in the patients responding to or failing therapy. In both MPA arms, plasma cortisol levels were suppressed. The drop in plasma cortisol levels was more pronounced in patients with objective remissions than in patients who failed ($P = 0.04$). In conclusion, oral and im MPA in the given doses had similar activity. Im administration of MPA should be reserved for patients not able to take oral medication. [Cancer Treat Rep 71:1151-1156, 1987]

In normal breast and endometrium, progesterone antagonizes the proliferative activity of estradiol by inhibiting the replenishment of estradiol receptors (ER) and by increasing the metabolism of estradiol to estrone (1). At pharmacological levels, some progestational agents, such as medroxyprogesterone acetate (MPA) or megestrol acetate, may also bind to androgen receptors (1,2). Very high doses of progestins may block the pathway of glucocorticoids at the receptor level and will also suppress adrenal cortical activity and have antigonadotropin effects (1,3). These mechanisms of action may be involved when progestins are used for patients with hormone-dependent breast or endometrial cancer. Furthermore, high levels of MPA at least may have a direct cytotoxic effect for those tumors (1). In patients with advanced breast cancer, the cumulative response rate to treatment with MPA increases with increasing doses (4). Daily doses of ≥ 500 mg of MPA have been advocated and have been investigated most extensively when the drug was given by im injection (5). In recent studies, comparable therapeutic effects during oral therapy with very high doses of MPA have been reported (6-10). In

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view of differences in the pharmacokinetic behavior of oral and im administered MPA (11–14) and to avoid eventual unnecessary im treatment, a randomized trial comparing the results and side effects of both routes of administration in patients with advanced breast cancer seemed justified. In most treatment strategies, high doses of MPA are given as second-line or later endocrine therapy. Therefore, to obtain maximal practical clinical information, this trial has been done in postmenopausal patients pretreated with at least tamoxifen and/or chemotherapy.

PATIENTS AND METHODS

Seven institutions were involved in this study. Postmenopausal patients with advanced breast cancer and measurable or evaluable disease that was refractory to treatment with tamoxifen and/or chemotherapy were eligible for this trial. Life expectancy had to be >3 months. Patients previously treated with progestins or corticosteroids were excluded. After stratification for ER activity of the tumor, duration of disease-free interval (DFI), and the dominant location of disease, patients were randomized to receive either 500 mg of MPA daily im for 28 consecutive days and 500 mg $2\times$ weekly thereafter to maintain the MPA levels obtained, or 300 mg of MPA orally $3\times$ daily. From the kinetics studies of Hesselius and Johansson (12), it was estimated that daily oral doses of about twice the im dose of MPA should give comparable serum MPA levels.

Therapy was planned to continue for at least 2 months if the disease continued to progress or, in case of an objective response, until relapse. Patients were evaluated after 2–3 months and every 3 months thereafter, according to International Union Against Cancer response criteria (15). All case records were reviewed extramurally. Measurements of ER activity in primary or metastatic tumor tissue were done in different laboratories by the same method (dextran-coated charcoal and analysis by Scatchard plot). Cytosols showing high-affinity binding of >10 fmol/mg of protein were considered ER positive (16).

Plasma MPA levels were measured sequentially in unselected patients at one institution by a radioimmunoassay (RIA). In brief, 250 μl of plasma was incubated with 10,000 dpm of [^{3}H]MPA as the recovery tracer and extracted with 5 ml of petroleum-ether at 40°C–60°C. The extract was dissolved in phosphate-gelatin buffer and used for the RIA. Intra- and interassay variations were 3.8% and 8.1%, respectively. The sensitivity of the assay was 3 pg/tube or 0.26 nmol/L (17).

Serial estimations of plasma cortisol were done initially in 94 patients before and during MPA therapy. Blood samples were taken between 10 AM and 2 PM in 85% of cases. The duration of remission and the survival time functions were calculated by the method of Kaplan and Meier (18). The differences between such functions were tested by the nonparametric test developed by Gehan and Mantel as described by Gehan (19). Further statistical analysis of data was performed by the chi-squared test for comparing percentages and Wilcoxon's two-sample test for comparing sample distributions of both treatment groups.

Confidence limits for the difference in response rates and for the ratio of median duration of response and median survival of oral and im treated patients were calculated by the methods indicated by Simon (20).

RESULTS

Between July 1981 and January 1984, 123 patients were randomized. Five patients were not eligible because of the lack of disease parameters. Eleven patients were not evaluable. Reasons for nonevaluability were early death (within 6 weeks, $n = 8$), incomplete data ($n = 1$), or protocol violation ($n = 2$). Half of those 16 patients were excluded after extramural review of case histories. The groups of 56 orally and 51 im treated evaluable patients did not differ significantly with regard to age, dominant location of metastasis, extent of advanced disease, previous therapy, or ER activity. However, a marginally significant difference in the number of patients without a DFI existed (four of 56 in the oral and 12 of 51 patients in the im group) ($P < 0.06$) (table 1). Of 107 patients, 25 (23%) achieved an objective remission. Furthermore, in 16 patients (15%) previous progressive disease remained stable for at least 3 months. Response rates did not differ significantly in orally and im treated patients (table 2). The median duration of response (objective remissions plus stable disease) in orally and im treated patients was 9 and 12 months, respectively (ranges, 3–28 and 3–31 months, respectively; $P > 0.1$; approximate 90% confidence interval im/oral, 0.38–1.27). The median duration of objective remissions only for orally and im treated patients was 12 months (range, 5–24+ months) and 14 months (range, 5–30+ months), respectively ($P > 0.10$).

Prognostic Factors and Response to Therapy

Table 1 gives information about the possible influence of clinical characteristics on the response to therapy (objective remissions versus total). Between the groups of orally and im treated patients, no relevant differences were found. Although not always statistically significant at the 5% level, the best treatment results were obtained in patients >50 years of age, in patients with a DFI of >1 year, and in patients presenting with advanced disease at the time of initial diagnosis. Furthermore, patients who had been treated previously with chemotherapy did worse than those treated with endocrine therapy only. Patients with ER-positive and ER-negative tumors had an objective remission in 28% and 7% of cases, respectively. In fact, all patients with ER-negative tumors had received previous chemotherapy. Finally, table 1 shows that treatment results were hardly influenced by the dominant location or extent of disease.

TABLE 1.—Prognostic factors and response to treatment*

Parameter	Oral ($n = 56$)	Im ($n = 51$)	All ($n = 107$)	P value of parameter
ER activity				0.12
Positive	7/30	9/29	16/59	
Negative	1/9	0/8	1/17	
Unknown	6/17	2/14	8/31	
Age (yrs)				0.02
< 50	1/21	2/16	3/37	
50–65	6/18	5/15	11/33	
≥ 65	7/17	4/20	11/37	
DFI (yrs)				0.16
None	0/4	5/12	5/16	
≤ 1	0/10	1/7	1/17	
> 1	14/41	5/32	19/73	
Dominant location of metastasis				0.96
Soft tissue	2/9	3/11	5/20	
Bone	6/30	6/24	12/54	
Viscera	6/17	2/16	8/33	
Extent of disease				0.40
Localized	1/2	2/6	3/8	
2–5 sites	2/10	2/16	4/26	
> 5 sites	11/44	7/29	18/73	
Previous therapy				0.004
Endocrine therapy	11/24	6/20	17/44	
Chemotherapy	0/4	0/6	0/10	
Chemotherapy + endocrine therapy	3/28	5/25	8/53	

*Values = No. of objective responses/No. of patients.

Duration of Survival

The median survival times from start of oral or im MPA therapy for all eligible patients were 12 months (range, 0.5–40) and 10 months (range, 0.5–41+), respectively ($P > 0.10$; approximate 90% confidence interval im/oral, 0.83–1.68). The median survival times from start of therapy for the patients responding (objective remission and stable disease) to oral or im MPA were 22 months (range, 4+–41) and 25 months (range, 6+–41+), respectively ($P > 0.10$; approximate 90% confidence interval im/oral, 0.43–1.64).

No significant differences in estimated survival times from detection of metastasis for all eligible orally and im treated patients were found. The median survival time from detection of metastasis for all treated patients was 39 months (range, 6–60+).

TABLE 2.—Results of therapy with MPA*

Result	Oral ($n = 56$)	Im ($n = 51$)	All ($n = 107$)
Objective remission†	14 (25)	11 (22)	25 (23)
Stable disease‡	9 (16)	7 (14)	16 (15)
Failure	33 (59)	33 (65)	66 (62)

*Values = No. of patients (%).
†$P > 0.10$; approximate 90% confidence interval oral/im, −10%–17%.
‡$P > 0.10$; approximate 90% confidence interval oral/im, −9%–14%.

Toxicity and Side Effects

With the exception of complications of im injections, the toxicity and side effects in both groups of patients did not differ significantly and are well-known from published data. The most striking effect was an increase in body weight in 54% and 67% of patients after 3 months of oral and im therapy, respectively, and continuing after 6 and 9 months, respectively. With increasing duration of treatment, cushingoid side effects developed in 19%, increasing to 46% of all patients. In two patients on im therapy, local infections developed after 9 months (15%). Thereafter, another two patients had the same complication (table 3).

Results of Measurements of Serum MPA and Serum Cortisol Levels

Mean serum MPA levels in nine orally and eight im treated patients reached values > 100 ng/ml and did not differ significantly. However, marked individual variations in MPA levels of both treated groups were found (fig 1A). Serum MPA levels in responding ($n = 6$) and failing ($n = 11$) patients also did not differ significantly (fig 1B). Plasma cortisol levels were about equally suppressed in orally and im treated patients (fig 2). After 3 months of therapy, patients with an objective remission and patients who failed had mean plasma cortisol levels of 0.05 ± 0.01 μmol/L and 0.14 ± 0.02 μmol/L, respectively ($P = 0.04$).

TABLE 3.—Side effects and toxicity by route of MPA administration*

Effect	Oral MPA			Im MPA		
	3 mos ($n = 56$)	6 mos ($n = 28$)	9 mos ($n = 16$)	3 mos ($n = 48$)	6 mos ($n = 24$)	9 mos ($n = 13$)
Increase in body weight	54	57	37	67	52	46
Cushingoid effects	16	36	50	23	54	42
Muscle cramps	20	29	12	19	33	23
Tremor	11	14	12	34	25	23
Edema	14	11	6	17	12	31
Perspiration	7	21	6	10	0	8
Hirsutism	5	7	12	4	12	23
Acne	0	4	0	4	4	15
Local infections (im group)	—	—	—	0	0	15

*Values = % of patients. Differences were not significant by the chi-squared test.

DISCUSSION

The overall objective remission rate in this trial was 23%. This is in accordance with the published results for high doses of orally or im administered MPA as second-line endocrine therapy in postmenopausal patients with advanced breast cancer (6,10,21,22). The overall objective remission rate was slightly higher than that of 17% found by Paridaens et al (23) in a study with a design comparable to ours. Results in orally or im treated patients did not differ significantly with regard to remission rate, duration of remission, or survival times. However, the corresponding 90% confidence limits for the ratios of the median duration of response and survival of oral and im treated patients indicate that the real differences of these values could be more pronounced. In both treatment arms, the best results were obtained in older patients not previously treated with chemotherapy. This is also in accordance with observations in the literature (8,22). In this study, 16 of 59 patients (27%) with ER-positive tumors had an objective remission, compared with only one of 17 (6%) patients with ER-negative tumors. Bumma (24) reported that 31 of 55 (56%) patients with ER-positive and seven of 42 (17%) patients with ER-negative breast cancer responded to high-dose MPA therapy. From the data of Cavalli et al (22), it can be seen that in a total of 46 patients, response rates to high-dose MPA therapy were 50% in the ER-positive and 30% in the ER-negative group. So, although our data and those in the literature suggest that ER activity is of predictive value for the results of MPA therapy, this correlation is less strongly marked than with other endocrine therapies. During extramural review, it became clear that the inclusion criterion of life expectancy of > 3 months was interpreted too optimistically for all eight patients who died within 6 weeks after beginning MPA therapy. All

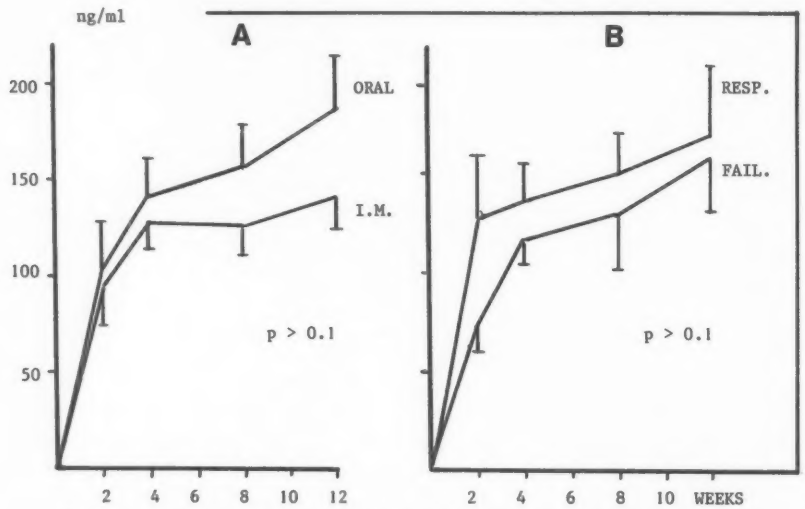

FIGURE 1.—(A) Mean ± SEM MPA levels in 9 orally and 8 im treated patients. (B) Mean ± SEM serum MPA levels in 6 patients responding to and 11 patients failing therapy.

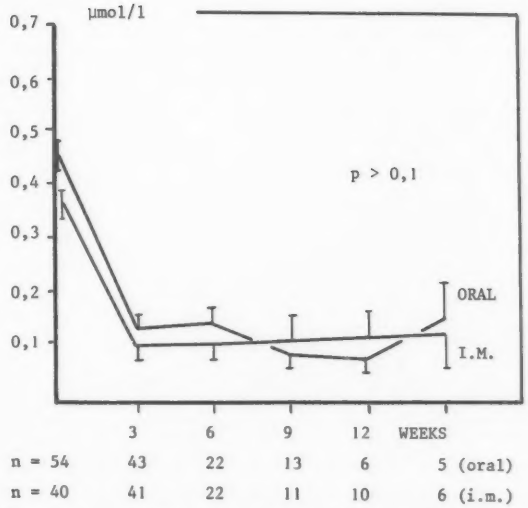

FIGURE 2.—Mean ± SEM plasma cortisol levels in patients treated with oral or im MPA.

those patients died from metastatic disease. Careful analysis did not disclose a direct causative connection between MPA therapy and early death. In none of the treated patients was an initial flare-up of the disease documented.

The doses of MPA administered, 900 mg orally daily and 500 mg im daily for 28 days, thereafter twice weekly, gave similar mean serum MPA levels, although individual variations were considerable. In accordance, Tamassia et al (13) and Panutti et al (14) showed that the daily oral dose of MPA should be two to three times higher than that given im to obtain similar steady-state plasma levels. After oral administration, MPA levels increase rapidly, whereas the terminal half-life is about 2 days. Only a small fraction (1%–10%) of the drug is absorbed from the gastrointestinal tract, so the bioavailability of orally given MPA is low. During im therapy MPA levels increase slowly due to the slow absorption rate of the drug, while the terminal half-life is very prolonged, about 6 weeks, as a result of the im depot effect (11–14). Marked individual differences in MPA levels in a steady-state situation of the same daily doses might be explained by differences in the absorption rate of MPA from the gastrointestinal tract or im depot and in differences in metabolic clearance of the drug. Although mean serum MPA levels were similar in patients who responded (objective remissions and stable disease, $n = 6$) and failed ($n = 11$), a small but significant difference in cortisol suppression in patients with objective remission and patients with progressive disease was found. This is in accordance with the data of Wander et al (25). Cortisol suppression did not differ in orally or im treated patients (fig 2). It has been shown by van Veelen et al (26) that suppression of plasma cortisol levels by MPA in the doses used in this trial is a result of combined effects of the drug or its metabolites on the pituitary gland and adrenal cortex. Adequate suppression of adrenal steroid synthesis in patients treated with MPA may have therapeutic consequences.

The side effects of both treatment strategies (table 3) are well known in the literature (4,8,22) and, except for the complications of im administration, were equally divided between both treatment arms. It must be stressed that the anabolic effect of high doses of MPA might be detrimental in already overweight breast cancer patients with bone metastasis.

In conclusion, high doses of MPA, given as a second- or late-line endocrine therapy for patients with advanced breast cancer, resulted in an objective remission rate of 23%. Because of differences in bioavailability of oral and im administered MPA, daily oral doses of about twice the im doses give rise to equivalent serum MPA levels and suppression of plasma cortisol levels. At the given doses, both treatment strategies had similar activity. Im administration of MPA should be reserved for patients not able to take oral medication.

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Preclinical Toxicology Studies of 4-Ipomeanol: A Novel Candidate for Clinical Evaluation in Lung Cancer[1,2]

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4-Ipomeanol (ipomeanol) is being developed as a potential antitumor agent to treat lung cancer. Ipomeanol produced a dose-related toxicity in $CD2F_1$ mice, Fischer 344 rats, and beagle dogs. The LD_{50} in mice after a single iv dose of ipomeanol was 35 mg/kg in males and 26 mg/kg in females. Minimal cumulative toxicity occurred in mice after seven doses; LD_{50} was 30 mg/kg/day in males and 21 mg/kg/day in females. In rats, iv doses $\geq$ 15 mg/kg were lethal. Labored respiration, terminal bronchiolar epithelial necrosis, interstitial inflammation, and alveolar edema were present in rats dosed with ipomeanol at $\geq$ 9 mg/kg. In addition to pulmonary lesions, splenic and thymic lymphocyte depletion and/or necrosis was present. Ipomeanol had little cumulative toxicity in rats given seven daily doses. In dogs, iv doses > 12 mg/kg were lethal. Dogs treated with lethal doses of ipomeanol showed rapid, shallow respiration and pulmonary edema prior to death; diffuse pulmonary congestion or hemorrhage and diffuse renal congestion were present at necropsy. Pulmonary microscopic changes caused by nonlethal doses of ipomeanol included subacute interstitial inflammation and necrosis of respiratory bronchiolar and alveolar duct epithelium. In contrast to rodents, seven daily doses of ipomeanol were cumulatively toxic in dogs. The nonlethal pulmonary effects of ipomeanol were reversible in all three species. Tolerance to lethal doses of ipomeanol occurred in animals of all three species pretreated with multiple nontoxic doses of the drug. The LD_{50} of ipomeanol in male and female mice increased 2.4- and 4.5-fold, respectively, in tolerant mice. In rats and dogs, previously lethal doses of 48 and 24 mg/kg were nonlethal after tolerance was induced by pretreatment with seven daily doses of ipomeanol. [Cancer Treat Rep 71:1157-1164, 1987]

Lung cancer is one of the most common and devastating of human neoplastic diseases. The increased incidence of lung cancer over the past 30 years has been linked to smoking as well as increased industrialization (eg, pollution and occupational hazards). Currently available chemotherapeutic agents are not generally useful in the treatment of pulmonary neoplasms. Therefore, the development of new antineoplastic agents selective for pulmonary tissue is necessary to effectively treat lung cancer (1,2).

4-Ipomeanol (ipomeanol), a naturally occurring furan, is a potent and selective pulmonary cytotoxin in several animal species (3,4). This compound was first isolated from sweet potatoes infected with the common fungus *Fusarium solanae* (5). Extensive studies have been conducted to explore the mechanisms of action and subsequent development of the lung injury caused by ipomeanol. In vivo administration of ^{14}C-labeled ipomeanol results in a high concentration of radioactivity bound to cellular macromolecules in the lung (3,4-8). The highly reactive metabolite, which binds to cellular macromolecules, is produced by the pulmonary mixed-function oxidase system. Autoradiographic studies have demonstrated that covalently bound ipomeanol metabolites are primarily located in the pulmonary bronchiolar Clara cells (8). Clara cells serve as stem cells for bronchial and bronchiolar epithelium (9) and are also a major source of mixed-function oxidase activity in the lung (8).

Studies by Falzon and co-workers (10) have shown that ipomeanol is selectively activated to reactive me-

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tabolites by human tumor cell lines with epithelial cell characteristics. The activation of ipomeanol to reactive metabolites, which covalently bind to cellular macromolecules, was directly related to the cytotoxicity of ipomeanol in human tumor cell lines of epithelial origin. Ipomeanol was not metabolically activated and did not demonstrate any cytotoxicity in human tumor cell lines of small cell origin.

Therefore, a proposal and rationale have been presented for clinical evaluation of this agent against human lung cancer (8,10). The purpose of the present study was to provide preclinical toxicological evaluation as a precedent for phase I clinical trials.

EXPERIMENTAL PROCEDURES

Drug Preparation

Ipomeanol was supplied by the National Cancer Institute (NCI). The drug was diluted to the appropriate concentration for dosing with 0.9% sodium chloride for injection. Ipomeanol was administered iv in all of these studies.

Mouse Lethality Studies

$CD2F_1$ mice were supplied by Charles River Breeding Laboratories (Portage, MI) through the Animal Genetics and Production Branch, Developmental Therapeutics Program, Division of Cancer Treatment, NCI. In the single-dose lethality study, 80 male and 80 female mice were assigned to eight groups with ten mice per dose-sex group. These dose groups received 35, 30, 25, 20, 15, 10, 5, and 0 mg/kg of ipomeanol. In a second study, ten male mice per group received 70, 60, 50, 45, 40, 35, or 15 mg of ipomeanol/kg, and ten female mice per group received 35, 30, 25, or 15 mg/kg. The study was terminated on Day 29.

In the lethality study using seven daily doses, ten mice per sex were assigned to seven dose groups and a vehicle control (VCTL) group. Doses for males were 70, 60, 50, 40, 30, 20, 10, and 0 mg/kg/day. Doses for females were 30, 25, 20, 15, 10, 5, 2.5, and 0 mg/kg/day. Doses were administered iv once daily for 7 consecutive days. The study was terminated on Day 35. Probit analyses were used to calculate the estimated LD_{90}, LD_{50}, and LD_{10}, and the 95% confidence interval (11).

Toxicity Studies

Toxicity was evaluated after ipomeanol treatment by monitoring clinical signs daily and evaluating hematology (hemoglobin, hematocrit, and rbc, total and differential wbc, and reticulocyte counts) and clinical chemistry parameters (BUN, SGOT, SGPT, serum glucose, and creatinine). In dogs, additional clinical chemistry parameters (alkaline phosphatase, prothrombin time, total protein, and sodium, potassium, calcium, chloride, and magnesium levels) were evaluated. Necropsy with complete gross and microscopic examination was conducted of all organ systems of dogs and major organ systems of rats.

Dog Toxicity Studies

Beagle dogs were supplied by the Developmental Therapeutics Program, NCI. In the single-dose study, dogs were dosed at the following levels: 48 mg/kg (one female); 24 mg/kg (one male); 12 mg/kg (three male and two female); 6 mg/kg (three male and three female); and 0.3 mg/kg (three male and three female). The dogs were necropsied on Days 8, 30, 58, and 61.

In the toxicity study using seven daily doses, four dogs (two male and two female) were dosed with 4.5, 0.3, or 0 mg/kg/day (VCTL). Each dog received a single iv dose of ipomeanol or vehicle on 7 consecutive days. One sex pair from each dose group was necropsied on Day 10, and the remaining dogs were necropsied on Day 64.

Rat Toxicity Studies

Fischer 344 rats were supplied by the Developmental Therapeutics Program, NCI. In the single-dose toxicity study, rats were treated at the following dose levels; one male and one female each with 48, 24, 12, 6, and 3 mg/kg and ten males and ten females each with 15, 9, and 0 mg/kg. Rats in the range-finding study were necropsied on Day 8. In the toxicity study, one-half of the surviving rats in each dose group were necropsied on Day 4, and the remaining rats were necropsied on Day 29.

The toxicity study using seven daily doses consisted of ten rats per sex treated with ipomeanol iv at 9.0, 4.5, and 0 mg/kg/day for 7 consecutive days. One-half of the animals in each dose group were necropsied on Day 11, and the remaining animals were necropsied on Day 37.

Tolerance Studies

The induction of tolerance to ipomeanol toxicity was evaluated in $CD2F_1$ mice, Fischer 344 rats, and beagle dogs after a pretreatment period of seven consecutive daily iv doses of ipomeanol. Pretreatment was followed by administration of a large toxic/lethal challenge dose of the drug. Mice were pretreated with iv injections of 10 mg/kg/day for 7 consecutive days. After pretreatment, ten mice of each sex received ipomeanol at the following doses: males received 163.5, 130.8, 98.1, 65.4, 32.7, and 0 mg/kg, and females received 114.5, 91.6, 68.7, 45.8, 22.9, and 0 mg/kg. The study was terminated on Day 29.

Dogs were pretreated with 1.5 mg/kg/day of ipomeanol for 7 consecutive days (Days −7 to −1). On Day 1, the dogs received challenge doses of 48 mg/kg (one male and one female); 24 mg/kg (one male and one female); 12 mg/kg (two male and two female); and 0 mg/kg (one male and one female). One male and one female in the group that received 12 mg/kg and in the VCTL group were necropsied on Day 8. All other animals were necropsied on Day 58.

Rats were pretreated with 4 mg/kg/day of ipomeanol for 7 consecutive days (Days −7 to −1). Challenge doses of 48, 24, 15, and 0 mg/kg were administered on Day 1. One-half of the rats (three male and three female) in each dose group were necropsied on Day 4, and the remaining rats were necropsied on Day 30.

RESULTS

The LD_{50} of a single iv dose of ipomeanol was estimated to be 35.0 mg/kg in male mice and 26.1 mg/kg in female mice (table 1). However, there was little cumulative toxicity of ipomeanol in mice, since the LD_{50} of seven daily doses of drug was 29.6 mg/kg/day for male mice and 20.7 mg/kg/day for female mice. In both studies, female mice were much more sensitive to the lethal effects of ipomeanol than male mice.

Single iv doses of ipomeanol > 12 mg/kg were lethal to Fischer 344 rats (table 2). A dose of 15 mg/kg resulted in 60% and 10% mortality in male and female rats, respectively. Signs of ipomeanol-induced toxicity in rats were the result of pulmonary compromise, characterized by labored respiration and nasal discharge and accompanied by decreased activity and decreased body weight. The toxicity caused by ipomeanol treatment was dose-related, with clinical signs of toxicity evident after a dose of 6 mg/kg, and toxicity became more severe with increasing doses. In contrast to the pattern in mice, male rats were more affected than female rats by the toxic and lethal effects of ipomeanol. Histopathologic lesions in the lungs of ipomeanol-treated rats included terminal bronchiolar epithelial necrosis, interstitial inflammation, and alveolar edema. The interstitial inflammation was characterized by increased thickness of the alveolar wall due primarily to increased cell number and secondarily to edema. Additionally, rats treated with 15 mg of ipomeanol/kg had splenic necrosis or cellular lymphocyte depletion of the thymus and/or spleen. There was marked elevation of SGOT (349–800 IU/L) and SGPT (100–169 IU/L) 3 days after a dose of 15 mg of ipomeanol/kg, which suggests hepatocellular damage. However, the serum transaminase activities returned to control values in surviving rats on Day 8, and there was no evidence of hepatic microscopic lesions.

There was no apparent cumulative toxicity of ipomeanol in rats. The tolerance of rats to seven daily doses of 9 mg/kg of ipomeanol was similar to the tolerance of a single dose of 9 mg/kg (table 2). Drug-related microscopic lesions were limited to the lung and included interstitial inflammation. These lesions were reversible; they were only evident on Day 10 and were not evident in animals necropsied on Day 37.

Ipomeanol also caused a dose-dependent toxicity in beagle dogs (table 3). Clinical evidence of pulmonary toxicity occurred 2–6 days after ipomeanol administration. Signs of pulmonary toxicity included rapid, shallow respiration and were accompanied by decreased

TABLE 1.—Lethality of ipomeanol in $CD2F_1$ mice: probit analysis*

Defined dose	Estimated dose mg/kg	Estimated dose mg/m^2	95% confidence interval (mg/kg)
		Male	
Single dose			
LD_{90}	37.5	112.5	36.1–59.6
LD_{50}	35.0	105.0	32.7–36.8
LD_{10}	32.7	98.1	19.8–34.0
7 daily doses			
LD_{90}	35.3	105.9	31.8–65.8
LD_{50}	29.6	88.8	23.5–33.5
LD_{10}	24.9	74.7	10.6–28.0
		Female	
Single dose			
LD_{90}	29.8	89.4	28.3–33.3
LD_{50}	26.1	78.3	24.6–27.4
LD_{10}	22.9	68.7	19.9–24.3
7 daily doses			
LD_{90}	23.3	69.9	21.6–35.0
LD_{50}	20.7	62.1	18.9–23.2
LD_{10}	18.4	55.2	12.8–19.8

* Male and female $CD2F_1$ mice were given a single iv dose or 7 daily iv doses of ipomeanol. Deaths were recorded over the following 28-day period, and probit analyses were used to calculate LD_{90}, LD_{50}, LD_{10}, and 95% confidence interval (11).

TABLE 2.—Characteristics of ipomeanol toxicity in Fischer 344 rats*

	Single dose (mg/kg)	7 daily doses (mg/kg/day)
Dose	48.0	9.0
	24.0	4.5
	15.0	0.0
	12.0	
	9.0	
	6.0	
	3.0	
Mortality	> 12.0	None
Clinical signs		
Labored respiration	15.0, 9.0	
Nasal discharge	15.0	
Decreased activity	15.0	9.0
Decreased body weight	15.0	9.0
Clinical chemistry		
Increased SGOT/SGPT	15.0	None
Histopathology		
Lung		
Terminal bronchiolar epithelial necrosis	15.0, 9.0	
Interstitial inflammation	15.0, 9.0	9.0
Alveolar edema	15.0	
Spleen		
Lymphocyte depletion or necrosis	15.0	
Thymus		
Lymphocyte depletion	15.0	

* Fischer 344 rats were treated with a single iv dose or 7 daily iv doses of ipomeanol. Clinical signs were monitored daily; hematology and clinical chemistry parameters were evaluated weekly; and complete necropsy and histopathologic evaluation of major organ systems were conducted.

TABLE 3.—Characteristics of ipomeanol toxicity in beagle dogs*

	Single dose (mg/kg)	7 daily doses (mg/kg/day)
Dose	48.0	4.5
	24.0	0.3
	12.0	0.0
	6.0	
	0.3	
Mortality	> 12.0	None
Clinical signs		
Rapid, shallow respiration	≥ 12.0	4.5
Decreased activity	≥ 12.0	
Decreased food consumption	≥ 12.0	
Histopathology		
Lung		
Subacute interstitial inflammation	12.0	4.5
Respiratory bronchiolar epithelial necrosis	12.0	
Alveolar duct epithelial necrosis	12.0	

*Beagle dogs were treated with single iv dose or 7 daily iv doses of ipomeanol. Clinical signs were monitored daily; hematology and clinical chemistry parameters were evaluated twice weekly; and complete necropsy and histopathologic examination of all organ systems were conducted.

activity and decreased food consumption. Single iv doses of ipomeanol > 12 mg/kg were lethal; in these dogs, adverse clinical signs progressed to labored respiration and serous nasal discharge before death due to respiratory failure. Lethal doses of ipomeanol caused marked pulmonary edema, diffuse pulmonary congestion, and/or hemorrhage and diffuse renal congestion. Dogs given nonlethal doses of ipomeanol had histopathologic lung lesions described as subacute interstitial inflammation characterized by thickening of alveolar walls by edema and inflammatory cells in alveolar lumen (figs 1 and 2). Acute, severe necrosis of respiratory bronchiolar epithelium and alveolar ducts was also present (fig 3, left). Lesions present in dogs treated with ≤ 12 mg of ipomeanol/kg were reversible; no gross or microscopic lung lesions were present in dogs necropsied on Day 59 or 61 (fig 3, right).

There was evidence of cumulative toxicity in dogs treated with seven daily doses of ipomeanol (table 3). Pulmonary toxicity characterized by rapid, shallow respiration developed on Days 6–9 in dogs treated with 4.5 mg of ipomeanol/kg/day for 7 days. Subacute interstitial

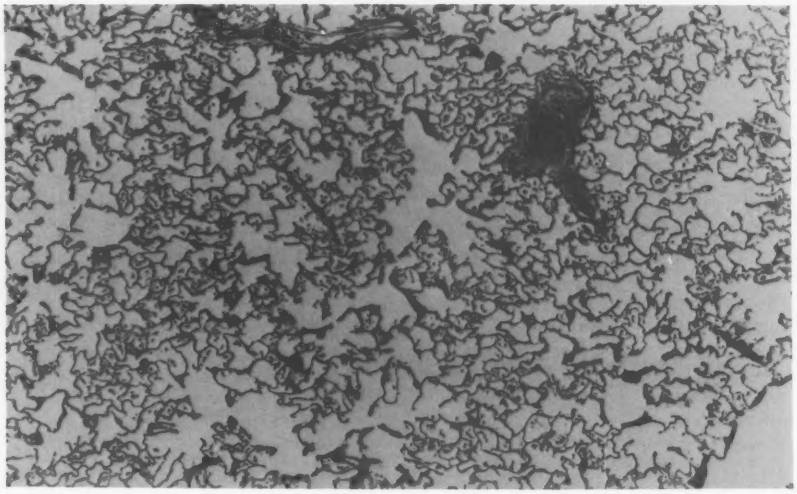

FIGURE 1.—Photomicrograph of lung from beagle dog treated with 12 mg of ipomeanol/kg (magnification, × 40). Alveolar interstitium is thickened and alveolar spaces contain cells.

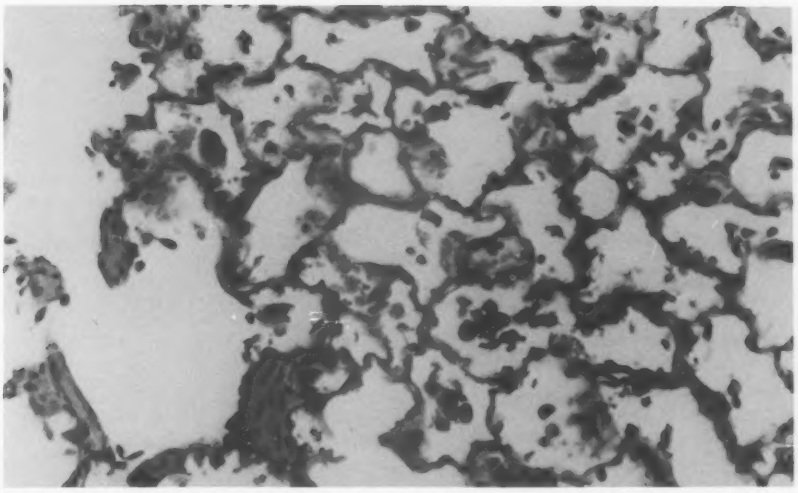

FIGURE 2.—Photomicrograph of lung from beagle dog treated with 12 mg of ipomeanol/kg shows more severely affected area than that in fig 1 (magnification, × 200). Most cells in alveolar spaces are macrophages.

inflammation was the only drug-related histopathologic lesion present at necropsy. Pulmonary lesions were reversible; no lesions were present in dogs necropsied on Day 64.

A dramatic tolerance to the toxic and lethal effects of ipomeanol occurs in both male and female mice. The LD_{50} in mice, after 7 days of pretreatment with 10 mg of ipomeanol/kg/day, was increased to 84.9 and 117.7 mg/kg in male and female mice, respectively (fig 4). This is a 2.4- to 4.5-fold increase in the LD_{50} after single doses of ipomeanol. Pretreatment of rats with 4 mg of ipomeanol/kg/day for 7 days prevented the lethal effects of the doses of 48, 24, and 15 mg/kg (table 4). The pulmonary toxicity of ipomeanol in tolerant rats was apparent histopathologically only after a challenge dose of 48 mg/kg, which is a lethal dose in nontolerant rats.

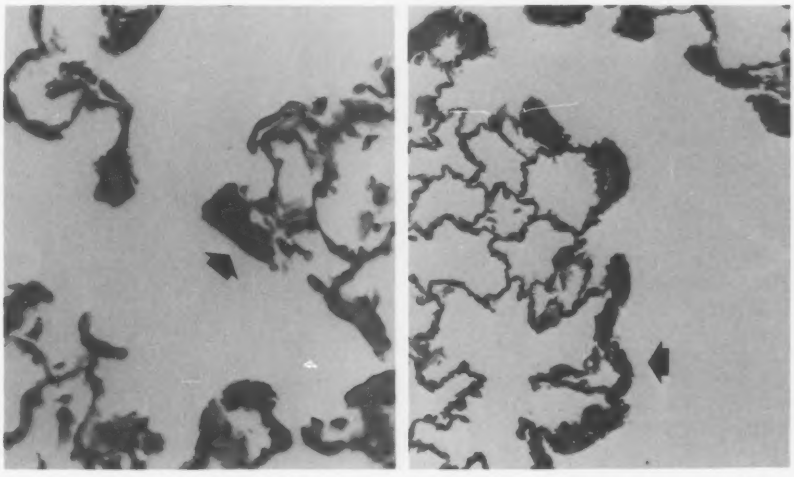

FIGURE 3.—Left: photomicrograph of respiratory bronchiolar/alveolar duct from beagle dog treated with 12 mg of ipomeanol/kg (magnification, × 200). Note loss of most of cuboidal epithelium (arrow); the few remaining cells are squamous and represent an attempt by remaining cells or regenerating cells to cover denuded area. Right: photomicrograph of respiratory bronchiole from beagle dog 58 days after treatment with 12 mg of ipomeanol/kg (magnification, × 200). Recovery has occurred; note intact low cuboidal epithelium lining bronchiole (arrow) and delicate alveolar walls.

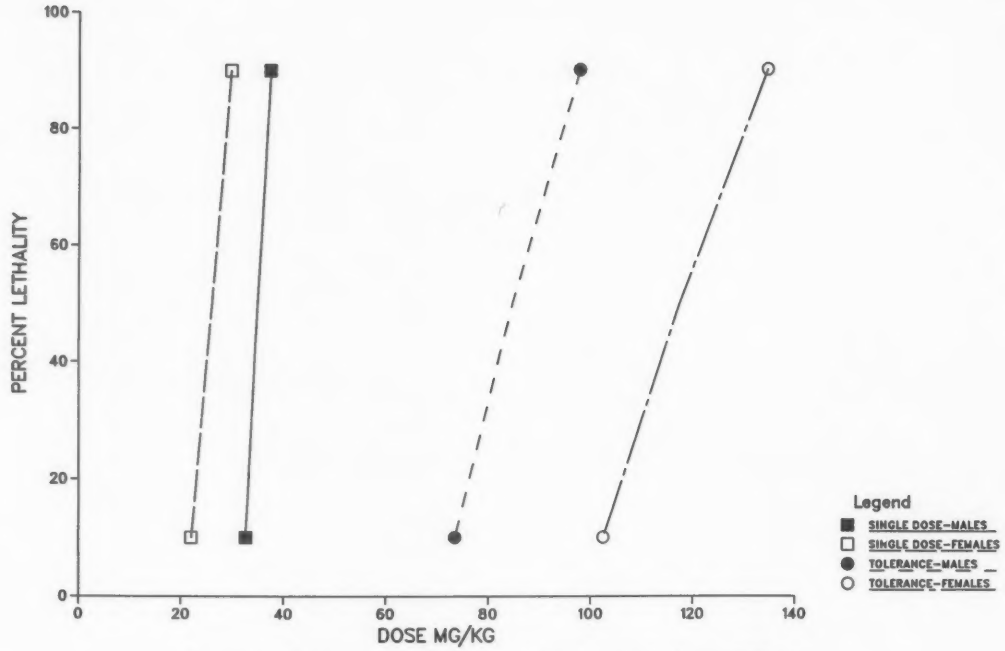

FIGURE 4.—Probit analysis of lethality data in nontolerant and tolerant $CD2F_1$ mice.

These pulmonary lesions were similar to lesions that occurred after single doses of ipomeanol and consisted of interstitial inflammation characterized by slight interstitial thickening due to increased cell number and edema fluid. Inflammatory cells were also present in the alveolar lumen. Similarly, previously lethal doses of ipomeanol of 48 and 24 mg/kg were not lethal to dogs after pretreatment with 1.5 mg/kg/day for 7 days (table 4). No ipomeanol-induced pulmonary lesions were present in tolerant dogs. A summary of the results of the effect of tolerance on ipomeanol toxicity in rats and dogs is presented in table 5.

TABLE 4.—Development of tolerance to ipomeanol toxicity in Fischer 344 rats and beagle dogs*

	Fischer 344 rats (mg/kg)	Beagle dogs (mg/kg)
Challenge dose	48.0	48.0
	24.0	24.0
	15.0	12.0
	0.0	0.0
Mortality	None	None
Clinical signs	None	None
Histopathology		
Lung		
Interstitial inflammation	48.0	None

*Fischer 344 rats were pretreated with 7 daily iv doses of 4.0 mg of ipomeanol/kg/day before administration of challenge dose. Beagle dogs were pretreated with 7 daily iv doses of 1.5 mg/kg/day before administration of challenge dose. Clinical signs were monitored daily; hematology and clinical chemistry parameters were evaluated weekly; and complete necropsy and histopathologic evaluation of all organ systems were conducted.

DISCUSSION

The present preclinical toxicology studies were designed to evaluate the safety and toxicity of single and multiple iv doses of ipomeanol as a precedent to clinical trials in cancer patients. In these studies, clinical signs were monitored closely; clinical pathology parameters were routinely evaluated numerous times during dosing and during the subsequent observation period; and complete necropsy and histopathologic examination of the organ systems were conducted twice during each study. Renal congestion was present in dogs given lethal doses of ipomeanol, but only pulmonary toxicity was present in dogs given nonlethal, toxic, single or multiple doses of ipomeanol. Rats had a transient elevation of serum transaminase activity, which is indicative of hepatocellular damage, but this was present only after highly toxic/lethal doses of ipomeanol. In addition, no microscopic lesions were present in the liver on Day 4; therefore, if ipomeanol caused hepatic damage, it was extremely mild and reversible. The lymphocyte depletion or necrosis of the spleen and thymus in rats given lethal doses of ipomeanol was most likely due to stress caused

TABLE 5.—Summary of development of tolerance to ipomeanol toxicity in Fischer 344 rats and beagle dogs

Species	Challenge dose (mg/kg)	Nontolerant	Tolerant
Fischer 344 rats	48	Lethal	Nonlethal, mildly toxic
	24	Lethal	Nonlethal, nontoxic
	15	Lethal/toxic	Nonlethal, nontoxic
Beagle dogs	48	Lethal	Nonlethal
	24	Lethal	Nonlethal
	12	Toxic	Nontoxic

by the pulmonary damage and, therefore, not directly drug-related. Other investigators have demonstrated that mature male mice given LD_{50} doses of ipomeanol developed renal cortical necrosis in addition to pulmonary lesions, while female mice, rats, rabbits, and guinea pigs only developed pulmonary lesions (12). Additionally, avian species, which do not have ciliated or nonciliated lining cells of the airway, do not develop pulmonary toxicity after ipomeanol but develop severe hepatotoxicity (13,14). It appears from these previous studies and the present studies that ipomeanol is primarily selective for pulmonary tissue in dogs and rats.

Pulmonary lesions resulting from ipomeanol administration were similar in both dogs and rats. Pulmonary interstitial inflammation was the primary lesion; bronchiolar epithelial necrosis became evident with increasingly toxic doses. The results of these studies are consistent with those of studies done in mice that demonstrated that ipomeanol caused necrosis of nonciliated bronchiolar Clara cells (8). Cells of the larger airways are affected by highly toxic and/or lethal doses of ipomeanol. In mice, massive pleural effusions and intra-alveolar and perivascular edema may be present after administration of highly toxic doses of ipomeanol (15). In the present studies, massive intra-alveolar edema occurred only after lethal doses of ipomeanol in rats and dogs, which were killed in a moribund state or died 24–72 hours after dosing. In both species, interstitial edema was present after nonlethal doses of ipomeanol. These lesions were reversible, however, and resolution of clinical signs of toxicity was complete within 2 weeks after dosing. Maximum ipomeanol-induced toxicity and/or lethality was seen within the first week after treatment in studies of both a single dose and seven daily doses in mice, dogs, and rats. There was no evidence of delayed toxicity or progression of pulmonary injury to fibrosis in dogs or rats treated with toxic but nonlethal doses of ipomeanol.

Rodents demonstrated a considerable sex difference in the occurrence of toxic effects from ipomeanol. Female mice were more sensitive than male mice to the lethal effects of ipomeanol. Conversely, male rats were more sensitive to the toxic effects of ipomeanol than female rats. Dogs did not demonstrate a marked sex difference in the toxic effects from ipomeanol. However, considering the relatively small number of dogs in each dose group and the nonlethal doses chosen, a subtle sex difference in the response of dogs to ipomeanol may not be apparent without increasing the dose or the group size.

The induction of tolerance to the pulmonary toxic effects of ipomeanol has been demonstrated in mice (16) (fig 4), rats (16) (table 4), rabbits (16), and dogs (table 4). It is conceivable that the "tolerance" phenomenon might be exploitable in clinical studies to protect the lungs from the effects of normally toxic doses of ipomeanol while maximizing the concentrations and potential cytotoxic effects of ipomeanol in lung tumor tissue. The tolerance phenomenon in mice, rats, and rabbits documented previously by Boyd and co-workers (16) showed that maximum tolerance was achieved after 7 days of pretreatment with ipomeanol once daily and that in male and female rats and male mice, the LD_{50} of ipomeanol was increased 2.5- to 3.3-fold. This is comparable to the 2.4- to 4.5-fold increase in the LD_{50} of ipomeanol in tolerant male and female $CD2F_1$ mice, respectively (fig 4) in this study.

The present studies, in addition, explored how the toxicity of ipomeanol was altered in tolerant animals. Tolerant rats treated with doses of ipomeanol that were lethal in nontolerant rats showed minimal signs of toxicity. The only lesions present were lung lesions that were similar histopathologically to the pulmonary lesions that occurred in nontolerant rats, albeit at much lower doses. Even more protection from the lethal and/or toxic effects of ipomeanol was observed in tolerant dogs. In dogs pretreated with 1.5 mg/kg/day for 7 days, ipomeanol-induced lethality was completely prevented, and in addition, there were no clinical signs of toxicity and no pulmonary histopathologic lesions present in tolerant dogs. However, dogs receiving challenge doses of 48 and 24 mg/kg after pretreatment were not necropsied until Day 59. Thus, the possibility of early microscopic changes with later resolution cannot be disregarded.

There is no evidence to suggest that tolerance to multiple small doses of ipomeanol develops in tumor tissue. Therefore, the development of tolerance in normal lung tissue could be advantageous in the clinical setting to help in maximizing the therapeutic efficacy of ipomeanol while minimizing its toxic effects on normal lung tissue.

In summary, the current studies show that the toxic-

ity of ipomeanol is highly specific for lung tissue. Single and multiple doses of ipomeanol produced widespread pulmonary damage in Fischer 344 rats and beagle dogs. In addition, tolerance to the toxic effects of ipomeanol can be induced in $CD2F_1$ mice, Fischer 344 rats, and beagle dogs by pretreating the animals with multiple small doses of ipomeanol.

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Phase I Clinical Investigation of Benzisoquinolinedione[1,2]

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A phase I study of benzisoquinolinedione (amonafide) was conducted in 30 patients with advanced solid tumors refractory to conventional therapy. The starting dose was 10 mg/m^2/day × 5 days and the highest tolerated dose was 625 mg/m^2/day × 5. The daily dose was mixed in 100 ml of normal saline and infused over 30–60 minutes. The dose-limiting toxicity was myelosuppression with nadirs of blood counts reached on Day 15 and recovery by Day 21–28. Other side effects included mild nausea and vomiting, mild phlebitis, skin rashes, and alopecia in some patients. A majority of the patients experienced dizziness, tinnitus, and hot flushes occurring predominantly at the higher dose levels. These were related to the rate of drug infusion and resolved on prolonging the infusion to 60 minutes. Pharmacokinetic studies of amonafide revealed a monoexponential plasma disappearance curve with a mean half-life of 3.5 ± 1.9 hours. The recommended dose of amonafide for phase II studies in solid tumors is 400 mg/m^2/day × 5 for good-risk and 300–320 mg/m^2/day × 5 days for poor-risk patients with courses repeated at 21–28-day intervals. [Cancer Treat Rep 71:1165–1169, 1987]

Benzisoquinolinedione (amonafide) is a novel synthetic compound which was originally synthesized by Brana and associates from Spain (1). The rationale underlying the development of this compound was to produce a single molecule containing chemical moieties belonging to three compounds with known cytotoxic properties. These included cycloheximide, an inhibitor of protein synthesis, aristolochic acid, and tilorone, an antiviral agent. The final product is an imide derivative of 3-nitro-1,8-naphthalic acid with a structure shown in figure 1. Amonafide was selected for clinical development based on its activity against mouse L1210 leukemia implanted ip. In four experiments, an increase in lifespan of 61%–106% with a mean value of 82% was obtained with administration of 16 mg/kg on Days 1–9. The drug was active over a twofold dose range, with the daily or intermittent administration demonstrating superior antitumor activity compared to the single-dose schedule (2). The maximal increase in lifespan in mice was observed when the drug was administered in a dose of 48 mg/kg on Days 1, 5, and 9. Amonafide demonstrated similar activity when it was given iv as compared to ip administration. Besides L1210, the drug showed significant activity against leukemia P388 and B16 melanoma. When tested against P388 resistant to DNA intercalators such as doxorubicin, mitoxantrone, and AMSA, there was complete cross-resistance with amonafide. The preclinical toxicology studies were conducted in mice and dogs (2). The LD10 in mice on the single-dose schedule was 182.4 mg/m^2 and on the 5-day schedule it was 75 mg/m^2/day. In the dog, the mouse LD10-equivalent dose (3.8 mg/kg/day × 5) resulted in no toxicity. However, on the single-dose schedule (9.2 mg/kg) there was evidence of mild hepatic and renal toxicity. A twofold increase in dose (18.4 mg/kg) resulted in signs of severe pain and/or neurotoxicity. Myelosuppressive toxicity in the form of leukopenia and mild anemia was observed with a dose of 3 × LD10 mouse equivalent, which amounted to 225 mg/m^2/day × 5 days. The starting dose in humans was based on the LD10 dose in mice (75 mg/m^2/day × 5), with one-tenth of the dose chosen for the first three patients. The major objective of this study was to define the maximal tolerated dose (MTD) of amonafide.

PATIENTS AND METHODS

A total of 30 patients with advanced solid tumors were treated with amonafide between April 1985 and June 1986. The general characteristics of the patients are summarized in table 1. All patients had received and failed standard chemotherapy. A majority of the

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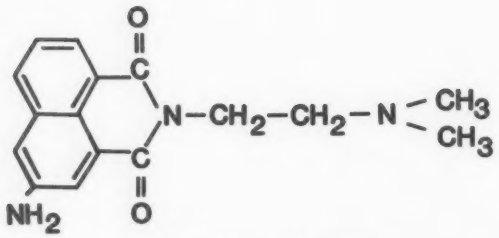

FIGURE 1.—Structure of amonafide.

patients had been treated with two or more chemotherapy regimens and nearly 50% of them had also received radiation therapy either postoperatively or for palliative therapy of metastatic disease. All patients had a serum bilirubin of < 1.5 mg/dl and a serum creatinine of < 1.5 mg/dl. All patients had normal peripheral blood counts prior to receiving amonafide and had not received any chemotherapy in the preceding 3 weeks. An informed consent was obtained indicating the investigational nature of this therapy.

Amonafide was obtained from the Investigational Drug Branch, National Cancer Institute, Bethesda, Maryland. It was supplied in a freeze-dried formulation containing 100 mg of drug in 6-ml flint vials. The lyophilized cake was dissolved in 2 ml of sterile water, yielding a 50-mg/ml red solution of the drug which was further diluted in 100 ml of normal saline. Since the reconstituted solution is not stable in 5% dextrose in water, this vehicle is not recommended for infusion of the drug. Amonafide was initially infused over a period of 30 minutes, but at the higher dose levels the infusion duration was prolonged to 1 hour because of acute toxicity associated with rapid administration.

Pretreatment patient evaluation included a cbc including differential and platelet count, a serum chemical survey including measurements of BUN, creatinine, bilirubin, SGOT, alkaline phosphatase, and CEA levels. A baseline urinalysis and an electrocardiogram were also obtained. The starting dose of amonafide was 10 mg/m^2/day $\times$ 5 days, repeated at 3-4-week intervals depending on recovery of blood counts. The dose escalation was flexible and involved approximately 100%–50% increments until the MTD was reached (table 2). A minimum of three patients were treated at each dose level and followed up for 3 weeks before escalating the dose to the next higher level and six or more patients were treated at the MTD. Following each course of amonafide, patients were monitored for toxicity on the hematopoietic system by measuring blood counts twice weekly and before each course of treatment. The serum chemical profile (SMA-12) was monitored once weekly including measurements of renal and hepatic function. An electrocardiogram and urinalysis were also repeated before each course of therapy. Tumor measurements were performed and recorded before each course of treatment and the x-rays and scans were repeated after two to three courses of therapy to monitor the changes in the tumor size. Pharmacologic studies were carried out in a limited number of patients to determine the kinetics of distribution and elimination of the drug.

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Entered	30
Median age in yrs (range): 51 (25-67)	
Sex	
Female	12 (40)
Male	18 (60)
Performance status	
0, 1	19 (63)
2, 3	11 (37)
Prior treatment	
Chemotherapy	30 (100)
Radiation therapy	18 (60)
No. of chemotherapy regimens	
1	11 (37)
2	11 (37)
≥ 3	8 (26)
Tumor types (*n*)	
Colon	9
Lung	9
Sarcomas	3
Melanoma	3
Head and neck	2
Breast	2
Unknown primary	2

RESULTS

A total of 83 courses of amonafide were administered to 30 patients in the dose range of 10–625 mg/m^2/day $\times$ 5. The dose escalation steps and the number of patients treated at each dose level are shown in table 2. No significant side effects were observed until the dose was increased to 260 mg/m^2 $\times$ 5 days. At the next dose escalation step (400 mg/m^2), significant toxicity was evident which led us to treat the largest number of patients

TABLE 2.—Phase I study of amonafide: dose escalation scheme

Dose level (mg/m^2 $\times$ 5 days)	No. of patients	No. of courses		
		Initial	Subsequent	Total
10	3	3	0	3
20	6	3	4	7
30	3	1	2	3
45	5	2	3	5
70	4	0	4	4
110	4	2	2	4
170	4	1	3	4
260	6	2	5	7
320	2	1	3	4
400	16	12	21	33
500	6	3	4	7
625	1	0	2	2

at this dose level both in order to define the variables affecting toxicity and for pharmacology studies. The dose of amonafide was increased to 500 mg/m^2/day × 5 in patients who had mild toxicity, whereas those experiencing severe toxicity were treated with a reduced dose of 320 mg/m^2. The myelosuppresive toxicity was severe at 500 mg/m^2 × 5 days except in one patient who had minimal toxicity and accordingly received 625 mg/m^2 × 5 days.

Hematologic Toxicity

The dose-limiting toxicity of amonafide was myelosuppression. Neutropenia and anemia were more pronounced with thrombocytopenia being less severe. A summary of myelosuppressive effects is shown in table 3. In a majority of patients an acceptable level of myelosuppression was achieved with 400 mg/m^2 × 5 days. However, for patients with extensive prior chemotherapy and particularly radiation therapy to the bones, this dose of amonafide was excessive and in two such patients a reduced dose level of 320 mg/m^2 also resulted in significant myelosuppression. Seven of 16 patients treated with 400 mg/m^2 × 5 days developed neutropenia of < 500/mm^3 as did four of six patients at the dose level of 500 mg/m^2 × 5 days. The neutropenia was complicated by fever requiring antibiotics in three patients at the 400-mg/m^2 dose and in four patients at the 500-mg/m^2 dose. Thrombocytopenia of < 100,000/mm^3 was observed in eight of 16 patients at the 400-mg/m^2 dose schedule. The platelet count was < 20,000/mm^3 in two patients at this dose level and in three of six patients at the 500-mg/m^2 dose range. All five of these patients were given prophylactic platelet transfusions and none of them had any bleeding complications. The myelosuppressive toxicity resulted in nadir blood counts by Day 14 or 15 and the counts recovered to the normal level by Day 21 in a majority although patients with severe neutropenia required 28 days. Although myelosuppression was intense, it was short-lived and not cumulative on repeated drug administration over a range of four to 11 courses.

Nonhematologic Toxicity

The other side effects associated with administration of amonafide are summarized in table 4. Except for some venous irritation and occasional nausea, no other side effects were observed in the dose range of 10–260 mg/m^2 × 5 days. In the dose range of 400–500 mg/m^2, most patients experienced a peculiar set of symptoms consisting of dizziness, a feeling of intense warmth resulting in diaphoresis, often accompanied by tinnitus and fullness of the ears. This effect was clearly related to the rate of drug administration and was largely ameliorated by extending the duration of drug infusion to 1 hour. The symptoms usually started near the end of the infusion and lasted for 1–2 hours and were aggravated if the patient got up and walked soon after the infusion. There was no postural hypotension to account for these symptoms. The next most frequent side effect was nausea and mild vomiting which was short-lived and easily controlled with antiemetics. A maculopapular rash was observed in several patients, particularly at the higher dose levels, but required no specific therapy. A mild to moderate degree of phlebitis was observed in some patients although this may be an underestimate since nearly 50% of the patients received the drug through the central venous catheter. A single case of perivenous drug infiltration occurred but was not associated with tissue necrosis. Alopecia was observed in some of the patients treated with the dose level of 500 mg/m^2 but was a minimal problem at the lower dose levels.

Antitumor Effects

Among the 25 patients evaluable for response, none achieved a complete or partial response. However, four patients evidenced some degree of therapeutic effect.

TABLE 3.—Hematologic effects of amonafide

Dose level (mg/m^2 × 5 days)	No. of evaluable courses	Median lowest counts × 10^3/mm^3	
		Absolute granulocytes (Day) (range)	Platelets (Day) (range)
10–170	30	4.0 (12) (2.2–10.6)	309 (11) (197–572)
260	7	3.7 (17) (0.8–15.6)	251 (15) (131–560)
320*	4	0.4 (14) (0.0–1.0)	52 (18) (10–86)
400	33	0.8 (15) (0.0–4.3)	124 (14) (5–509)
500	7	0.3 (15) (0.0–2.3)	103 (15) (6–239)
625	2	1.5 (14) (1.3–1.7)	197 (13) (191–203)

*Dose level arrived at after observation of excessive toxicity at the 400-mg/m^2 level in patients with extensive prior treatment.

TABLE 4.—Nonhematologic toxic effects of amonafide*

Toxic effect	Dose level (mg/m^2)						Total
	10–170	260	320	400	500	625	
Evaluable courses	30	7	4	33	7	2	83
Nausea and vomiting	1	2	3	24	5	1	36
Dizziness	—	1	—	18	5	1	25
Diaphoresis	—	—	—	10	2	—	12
Phlebitis	7	—	—	5	—	—	12
Alopecia	—	—	—	7	4	—	11
Skin rash	1	1	—	5	2	—	9
Tinnitus/ear fullness	—	—	1	6	1	—	8
Malaise	—	—	—	4	3	1	8
Stomatitis	2	—	—	1	—	—	3
Dyspnea	—	—	—	2	—	—	2

*Values = No. of courses.

These included two patients with lung cancer whose disease stayed in control for 8 months and 10 months, respectively. One patient had bronchoalveolar carcinoma with diffuse infiltrates in both lungs which resolved completely, although his tumor in the lymph nodes remained unchanged for 8 months. The second patient had squamous cell carcinoma of the lung with unmeasurable disease which was unchanged for a period of 10 months. One patient with squamous cell carcinoma of the esophagus had no change in his tumor for 5 months and another with adenocarcinoma of the lung had stable disease for 5 months.

Pharmacokinetics Studies

The plasma kinetics and urinary excretion of amonafide were studied in seven patients treated with a dose of 400 $mg/m^2 \times 5$ days. The drug concentrations in plasma and urine were determined using reversed phase, high-pressure liquid chromatography (3). Amonafide was eliminated in a first-order, monoexponential fashion with a mean half-life of 3.5 ± 1.9 (SD) hours. The mean volume of distribution was 151.2 ± 105.2 (SD) L/m^2. Total body clearance had a mean value of 28.9 ± 7.8 (SD) L/m^2/hour with renal excretion accounting for approximately 23% of the drug (4). The renal excretion, however, was highly variable and ranged from 5% to 45% of the dose during the initial 24 hours following drug administration. A number of amonafide metabolites have been identified in dogs and humans, although their contribution to its cytotoxicity is yet undetermined (4,5).

DISCUSSION

This is the first report of clinical experience with amonafide. The drug was generally well tolerated and produced myelosuppression as its dose-limiting toxicity. The myelosuppression was predictable, reversible, and noncumulative as shown by several patients treated with multiple courses. Rapid administration of the drug produced lightheadedness, diaphoresis, and a sensation of "plugged ears" often accompanied by tinnitus. These symptoms were worse if the patient attempted to get up and walk around and generally disappeared within 30–60 minutes of drug administration. The symptoms could be ameliorated by slowing the rate of infusion to 1 hour at the dose levels of 400 mg/m^2 or higher. The gastrointestinal effects were relatively mild, usually consisting of transient nausea with occasional vomiting. Although there were a few instances of stomatitis, the lack of dose-response effect suggests that they were probably not drug related. Another remarkable feature of amonafide was its relative lack of toxicity on hair follicles with a majority of the patients having minimal or no significant hair loss. However, patients treated at the higher dose levels (500 $mg/m^2 \times 5$ days) tended to experience partial or complete alopecia. Interestingly, a single patient treated at 625 $mg/m^2 \times 5$ days experienced no alopecia.

In this phase I study, it is not possible to ascertain the antitumor activity of amonafide. Nevertheless, two patients with lung cancer evidenced some antitumor activity. We believe that amonafide should be further investigated in phase II studies in solid tumors to further evaluate its antitumor activity. The drug should also be evaluated in patients with acute leukemia on account of its intense myelosuppression. A phase I study in leukemia patients starting amonafide at 600 $mg/m^2 \times 5$ days has recently been initiated at our institution. For phase II evaluation in patients with solid tumors an initial dose of 400 mg/m^2/day $\times 5$ days is suggested. Patients with compromised bone marrow due to extensive prior chemotherapy or radiotherapy should receive a lower initial dose of 300–320 mg/m^2/day. We recommend that this drug be mixed in 100–200 ml of normal saline and infused over 45 minutes to 1 hour. The courses of treatment can generally be repeated at 3-week intervals except in patients who experience severe myelosuppression where an interval of 28 days may be necessary.

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Phase I Trial of Taxol in Patients With Advanced Cancer[1,2]

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Taxol is a unique plant-derived antineoplastic agent that appears to exert its cytotoxic effect by interfering with microtubule structure and function. In this phase I trial, in which the drug was given as a brief iv infusion every 3 weeks, the dose-limiting toxicity was leukopenia, with thrombocytopenia being seen much less frequently. Sensory neuropathy was frequently seen at the highest dosage, with numbness and paresthesias appearing in a glove-and-stocking distribution. In some cases, this appeared to be a cumulative effect. Total alopecia was common. Other toxic effects observed included nausea and vomiting, mucositis, myalgias, and phlebitis. The frequent occurrence early in the study of acute cardiovascular and pulmonary toxicity suggestive of hypersensitivity reactions was decreased in frequency and severity by prolonging the infusions and premedication with corticosteroids and antihistamines. Two heavily treated patients appeared to respond to this agent, one with non-small cell lung cancer and one with ovarian cancer. Based on these data, we recommend a starting dose in phase II trials of 212 mg/m^2 in patients with minimal prior therapy and 170 mg/m^2 in heavily treated patients. At least initially, these trials should be carried out in institutions familiar with the use of this drug. [Cancer Treat Rep 71:1171-1177, 1987]

Microtubules are self-assembling and self-disassembling intracellular polymeric protein structures composed of tubulin monomers, whose function is crucial to living cells. Microtubule function has been primarily associated with the separation of the duplicated G_2 complement of chromosomes in the mitotic phase of the cell cycle. They are also important in the performance of vital interphase functions such as maintaining cell shape and conferring directionality to various aspects of cell motility and intracellular transport (1).

Taxol (fig 1) is a novel diterpene plant product isolated from the western yew, *Taxus brevifolia* (2). This compound has the unique effect in vitro of promoting the assembly of calf brain microtubules and stabilizing the tubulin polymers against depolymerization (3). Taxol binds with high affinity to polymerized tubulin and makes the affected microtubule structures unusually stable. This stability inhibits the dynamic process of reorganization of these structures that normally occurs in the intact cell (4). In the cell lines that have been studied, cellular replication appears to be inhibited at the G_2/M phase of the cell cycle, and indirect immunofluorescence studies with antitubulin antibodies demonstrate the formation of abnormal arrays or "bundles" of tubulin (5,6). These unique findings on the mechanism of action of this compound suggested that exploratory evaluation as an antineoplastic agent should be considered.

The preclinical evaluation of taxol demonstrated that the drug had activity against both murine solid tumor lines and leukemic cell lines. It had its greatest activity against ip B16 murine melanoma and the subrenal capsule implants of the MX-1 human breast cancer xenograft in nude mice. A lower level of activity was also seen in the ip P388 and L1210 murine leukemias and the CX-1 colon cancer and LX-1 lung cancer human xenografts (7). These encouraging results provided further rationale for clinical evaluation of this compound.

Preclinical toxicity studies were performed in several animal species, including mice, rats, and dogs (7). The toxic effects observed in the three species were quite consistent and were most evident in tissues with a high cell turnover. A principal toxic effect was dose-related, reversible myelosuppression. Dose-related lymphoid depletion was also seen in all species. Gastrointestinal toxic effects included anorexia, diarrhea, weight loss, emesis, and mucosal ulceration. Abnormalities were also seen in the reproductive system, with necrosis of devel-

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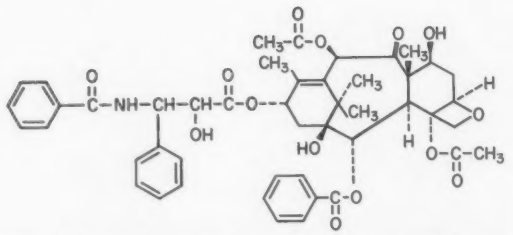

FIGURE 1.—Unique structure of the plant-derived antineoplastic agent taxol.

oping spermatocytes and seminiferous tubules. The reversibility of these lesions was not assessed.

The vehicle in which the drug was formulated was also shown to have inherent toxicity. Cremophor EL (polyoxyethylated castor oil) given in increasing doses, without taxol, resulted in vasodilation, labored breathing, lethargy, and presumed hypotension and death in some animals. Dogs appear to be sensitive to the cardiovascular effects of this agent, but the clinical studies were approached with great caution.

The toxic dose low (TDL) in dogs was 45 mg/m^2 given as a single dose. No murine data on this schedule were available because of the vehicular toxicity. The starting dose for this study was 1/3 of the TDL, or 15 mg/m^2.

The phase I trial of taxol at the Johns Hopkins Oncology Center had several objectives: (*a*) to determine the maximally tolerated dose of taxol which could be given iv as a single infusion every 21 days; (*b*) to describe and quantitate the clinical toxic effects of taxol; (*c*) to seek preliminary evidence of therapeutic activity of taxol in patients with advanced cancer; and (*d*) to investigate the pharmacology of taxol when given by short iv infusion. The detailed pharmacologic and pharmacokinetic studies which accompanied this trial were reported separately (8). When the study was initiated, we had planned to give the drug over 1 hour. Because of the acute toxicity which accompanied this short infusion, patients subsequently received the drug over 6 hours. The details of this change and the acute toxic effects encountered will be described further with the remainder of the results.

MATERIALS AND METHODS

Eligibility

Only patients with histologically diagnosed solid tumors which were refractory to conventional therapy or for which no effective therapy was known were candidates for entry onto study. Prior to entry all patients had a complete history and physical examination performed. Height, weight, performance status, and tumor measurements were recorded. Initial laboratory data obtained included a cbc, differential wbc count, platelet count, serum electrolytes, BUN, glucose, creatinine, total protein, albumin, calcium, phosphate, uric acid, alkaline phosphatase, total and direct bilirubin, SGOT, SGPT, and prothrombin time. Chest radiograph, electrocardiogram, and urinalysis were also done prior to therapy. Eligibility criteria also included age, 20–75 years; an Eastern Cooperative Oncology Group (ECOG) performance status of 3 or lower (able to perform minimal self-care); life expectancy of at least 4 weeks; no major surgery within 14 days and no chemotherapy or large-field radiotherapy within 28 days of entering onto protocol; adequate bone marrow function (wbc count $> 4000/mm^3$, platelet count $> 100,000/mm^3$), hepatic function (total bilirubin < 2.0 mg/dl), and renal function (creatinine < 1.5 mg/dl); no significant underlying cardiac disease because of the risk of acute cardiovascular side effects; and no other coexistent medical problems of sufficient severity to prevent full compliance with the study. All patients gave written informed consent according to federal, state, and institutional guidelines.

Dosage and Formulation

Taxol for clinical use was provided by the Pharmaceutical Resources Branch of the National Cancer Institute as a concentrated sterile solution, 6 mg/ml in a 5-ml ampoule, formulated in 50% polyoxyethylated castor oil (Cremophor EL) and 50% dehydrated alcohol, USP. The contents were diluted prior to use. The stability of the formulated product increased with dilution, with 0.6-mg/ml and 0.03-mg/ml solutions showing no appreciable loss in potency at 4 and 24 hours, respectively.

The starting dose for this study was 15 mg/m^2 iv as a single bolus, with escalations thereafter following a modified Fibonacci search scheme.

Drug Administration

Because of concern for the acute toxicity of this agent, all but a very few courses of taxol were administered on the inpatient units of the Johns Hopkins Oncology Center, with the infusions closely monitored by physician and nursing staffs and with facilities and equipment for cardiopulmonary resuscitation immediately available. The infusions were initially given for 1 hour, but because of a high incidence of acute hypersensitivity reactions of varying severity seen in this and other studies (9), the duration of infusion was increased and steroid and antihistamine premedications were given as described later. The last 14 patients were premedicated and were treated with 6-hour infusions in which the rate of infusion was started slowly and increased over the initial 2 hours to a rate allowing completion in 6 hours.

Follow-up Studies

Patients were seen weekly while on study, with history and physical examination performed along with the recording of weight and performance status. The routine laboratory studies noted above were also done at each visit. Toxicity and responses were graded and

TABLE 1.—Patient characteristics*

Characteristic	
No. entered	30
No. of courses/No. evaluable	71/67
Male:female	20:10
Median age in yrs (range)	54 (37–75)
Performance status (ECOG)	
0	2
1	19
2	7
3	2
Prior therapy	
Chemotherapy	7
Chemotherapy and radiotherapy	21
None	2
Tumor types	
Colon	8
Lung	5
Breast	3
Cervix	3
Head and neck	2
Melanoma	2
Vater's ampulla	1
Bladder	1
Ovary	1
Pancreas	1
Renal	1
Gastric	1
Unknown primary	1

*Unless stated otherwise, values = No. of patients.

evaluated by ECOG criteria (10). Radiographic evaluation of measurable disease was performed after every two cycles of chemotherapy.

RESULTS

Thirty patients were enrolled on study, and their characteristics are shown in table 1. These patients received 71 courses of taxol at the dosage levels shown in table 2. All but four courses were completely evaluable for toxicity, and all of these four were in patients who had the drug discontinued for acute reactions. Twenty patients received more than one course of taxol. Five patients received more than two courses each (three, four, five, nine, and nine courses). Five patients were treated at more than one dose level (table 2). One patient was escalated from 15 to 20 mg/m^2. Two were escalated from 170 to 212 mg/m^2. Two had dose reductions from 265 to 212 mg/m^2.

The major toxic effects associated with taxol administration are shown in table 3. Nausea and vomiting were mild and infrequently seen. Only one course below 212 mg/m^2 was associated with gastrointestinal symptoms. Five of 30 courses at 212 mg/m^2 were associated with nausea and vomiting (grade 2); three of these occurred in the same patient. One patient treated at 212 mg/m^2 developed symptomatic diarrhea. Mucositis was also infrequently seen. Three of 30 courses at 212 mg/m^2 caused grade 1 or 2 mucositis. In one patient treated at 265 mg/m^2, the gastrointestinal ulceration was severe and the extent of the mucosal barrier breakdown was life-threatening. Symptomatic sensory neuropathy was a common finding at the three highest doses. Seven of eight courses at 170 mg/m^2 were associated with mild to moderate paresthesias. All but one of the four patients were affected, and the patient who received four courses at this dose experienced recurrent symptoms with each dose and a gradually increasing residual effect. Twenty-three of 30 courses at 212 mg/m^2 resulted in paresthesias, with six of nine patients being affected, and at 265 mg/m^2, four of five courses resulted in neurotoxicity affecting all four patients treated. The pattern was again similar, with paresthesias occurring 1–3 days following drug administration, with gradual improvement thereafter. The two patients who received nine courses were both treated at the three highest doses, and both experienced increasing symptoms. One of these patients began to have significant difficulty with fine motor movements, suggesting a motor component to the neuropathy as well. Altogether, 18 patients were treated at the three highest doses. Of the 11 patients in this group who had received prior therapy with vinca alkaloids or cisplatin, nine developed symptomatic neuropathy. Seven patients had received no prior neurotoxic therapy, and five of these developed neuropathy. Whether prior therapy predisposes to this toxic effect will require further study.

Myelosuppression, principally leukopenia, was the dose-limiting toxicity in this study. As shown in table 3, thrombocytopenia was infrequent. All but one of the patients treated at the three highest doses encountered at least grade 1 leukopenia. Only the patient with grade 4 toxicity at 265 mg/m^2 required antibiotics during a febrile and granulocytopenic episode. The hematologic toxicity is demonstrated in table 4, in which the mean wbc count nadir and the range of nadir wbc counts are given and the percent decrease is expressed. Similar data are provided for platelet count nadirs. These data demonstrate a dose-dependent drop in both hematologic parameters, with wbcs affected to a greater extent. Since blood counts were routinely done only on a

TABLE 2.—Dose escalation

Dose (mg/m^2)	No. of patients* (No. evaluable)	No. of courses (No. evaluable)
15	3 (3)	7 (7)
30	3 (3) (1 patient)	3 (3)
60	3 (3)	4 (4)
90	4 (4)	7 (5)†
135	4 (4)	6 (5)†
170	5 (5)	9 (8)†
212	9 (9) (4 patients)	30 (30)
265	4 (4)	5 (5)

*5 patients were treated at more than 1 dose level, as indicated. Dose indicated is that at which patients received subsequent courses.

†Small fraction of dose delivered because of acute onset of allergic reactions.

TABLE 3.—Toxicity of taxol*

Dose (mg/m^2)	No. of courses	Toxicity grade	Nausea/ vomiting	Peripheral nerve	Mucositis	Leukopenia	Thrombocytopenia
15	7	0	7	7	7	4	7
		1	0	0	0	3	0
		2	0	0	0	0	0
30	3	0	3	3	3	2	2
		1	0	0	0	1	0
		2	0	0	0	0	1
60	4	0	4	4	4	4	3
		1	0	0	0	0	1
		2	0	0	0	0	0
90	6	0	6	6	6	2	6
		1	0	0	0	4	0
		2	0	0	0	0	0
135	5	0	5	5	5	3	4
		1	0	0	0	0	0
		2	0	0	0	0	1
		3	0	0	0	2	0
170	8	0	7	1	8	1	8
		1	1	5	0	5	0
		2	0	2	0	2	0
		3	0	0	0	0	0
212	30	0	25	7	27	0	28
		1	0	17	1	9	1
		2	5	6	2	12	0
		3	0	0	0	5	0
		4	0	0	0	4	1
265	5	0	5	1	4	0	4
		1	0	3	0	1	0
		2	0	1	0	1	0
		3	0	0	1	2	0
		4	0	0	0	1	1

*Values = No. of courses.

weekly basis, it is possible that lower counts were occasionally missed. In seven courses, it required more than 22 days for the wbc count to return to $> 4000/mm^3$. The infrequent platelet count nadirs occurred between Days 7–11 and recovered promptly. Anemia requiring transfusion was seen in only one patient.

The alopecia seen with taxol was unique in several respects. Of the 12 patients treated at doses of 15–90 mg/m^2 who had hair at the time of drug administration, only two developed alopecia, both at 90 mg/m^2. Nineteen patients received taxol at doses of 135–265 mg/m^2 who were evaluable for alopecia, and in 17 hair loss occurred. The loss of scalp hair occurred between Days 14–21 and was sudden and relatively complete, often occurring in a single day. Patients receiving multiple courses lost all body hair, including axillary and pubic hair, eyelashes, eyebrows, and the hair on extremities. The results for the small number of patients followed for sufficient periods after discontinuation of taxol suggest that this hair loss is reversible.

Several miscellaneous toxic effects were seen which appeared temporally to be drug related and should per-

TABLE 4.—Hematologic toxicity of taxol

		Wbc count			Platelet count		
			Nadir (10^3 cells/mm^3)			Nadir (10^3 cells/mm^3)	
Dose (mg/m^2)	No. of courses	Mean decrease (%)	Range	Mean	Mean decrease (%)	Range	Mean
15	7	7.7	3600–17,300	9160	16	173–286	251
30	3	16	3300–10,000	6130	40	89–324	221
60	4	18	4000–7500	5630	19	101–377	245
90	6	24	3400–4400	3940	19	143–321	231
135	5	40	1100–8100	4360	27	73–715	380
170	8	54	3200–4700	3960	19	263–413	305
212	30	64.7	1000–4100	2513	23	29–367	240
265	5	70	33–3100	1780	26	7–293	176

TABLE 5.—Acute reactions to taxol

Patient No.	Dose (mg/m^2)	Infusion time (hrs)	Course No.	Comments*
1	90	1	1	Urticaria over both antecubital fossae during first course. No toxicity during second course; antihistamine premedication given.
2	90	1	1	Life-threatening hypotension developed within 2 mins of initiation of first-course infusion. Multiple PVCs. Crampy abdominal pain. Required pressors and antiarrhythmics. No subsequent course.
3	90	1	2	Bronchospasm within 10 mins of second-course infusion. Infusion discontinued. No subsequent course.
4	135	1	2	Bronchospasm, shortness of breath, ventricular ectopy, and erythematous skin rash within minutes of starting second-course infusion. Infusion discontinued. No subsequent course.
5	135	1	2	Mild hypertension, rales, PVCs, and bradycardia during second-course infusion. Resolved after infusion. No subsequent course.
6	170	6	2	Bronchospasm, tachycardia, and diffuse erythematous rash within minutes of starting second course. Infusion discontinued. No subsequent course.
7	212	6	2	Erythematous rash over arms and chest during second-course infusion. Resolved after infusion. No subsequent course.
8	265	6	2	Diffuse pruritic, erythematous rash developed during infusion. Worse on Day 2. No subsequent course.

*PVCs = premature ventricular contractions.

haps be looked for in subsequent studies. One patient treated at 265 mg/m^2 complained of double vision for 1 week following taxol administration. Neurologic evaluation was unrevealing of any other etiology. Five courses in two patients resulted in local venous toxicity in the form of erythema, tenderness, and discomfort along the course of the injected vein. As this resolved, sclerosis and hyperpigmentation occurred. Three patients treated at the two highest doses by 6-hour infusion developed myalgias and arthalgias 2–3 days after the end of the taxol infusions. In two patients treated at 265 mg/m^2, this was a major symptomatic event. In two patients who received five and nine courses of taxol, it recurred with each course. The etiology of these complaints is not known.

A major concern in the initiation of this study was the possibility of acute cardiopulmonary toxicity or hypersensitivity reactions. Between the doses of 15 and 135 mg/m^2, when the drug was given over 1 hour, five reactions of varying severity were observed in 27 courses of therapy administered to 18 patients. These reactions are described briefly in table 5. Three of these episodes were of sufficient severity to warrant discontinuation of the infusion, and one was life-threatening and required aggressive support for several hours to prevent a more dire outcome. These data, along with those from other centers reporting a similarly high rate of acute reactions, prompted a change in the schedule of administration (9). Since two of the five acute episodes, including the most severe episode, occurred in patients receiving their first course of taxol, there was no definite indication that prior exposure was a risk factor for toxicity. The pathophysiology of these reactions and whether they were immunologically mediated could not be ascertained from the clinical data available. Namely, we could not be certain whether these were true anaphylactic reactions mediated by IgE or whether they were nonimmunologic phenomena such as those seen with radiocontrast infusions.

It was decided to proceed with the trial with a schedule of premedication modeled after that described for patients with a severe previous reaction to iv radiocontrast medium (11). Patients received 40 mg of prednisone orally or the steroid equivalent iv 14 hours before

drug administration and additional doses at 7 hours and immediately prior to drug administration. They were also given 50 mg of diphenhydramine and 300 mg of cimetidine iv 30 minutes prior to drug administration to block both classes of histamine receptors. The duration of infusion was also prolonged, as described in Materials and Methods. The final 14 patients on this study received 43 courses of taxol on this schedule with only three minor reactions (table 5). Only one of these episodes required discontinuation of drug. All occurred on the second exposure to taxol. Of the eight acute reactions observed, only patient 1 (table 5) was treated with a subsequent course of taxol.

There was evidence for antitumor effect in two patients. One patient with an adenocarcinoma of the lung had a CEA decrease from 28 ng/ml to 10 ng/ml and remained at that level for 7 months. He had no easily measurable disease clinically or radiographically. A second patient with epithelial ovarian cancer had a decrease in multiple measurable peritoneal masses on computerized tomographic scan and a disappearance of gross ascites for 5 months. During this time her performance status returned to normal.

DISCUSSION

Taxol belongs to a unique class of plant-derived compounds on the basis of both its structure and the putative mechanism by which it affects cells and exerts its cytotoxicity. The activity noted in experimental tumor systems provided added impetus for the clinical evaluation of this drug.

In this phase I study, the dose-limiting toxicity was leukopenia, with thrombocytopenia being an infrequent finding. The leukopenia was clearly dose related, with nadirs occurring between Days 8–13 and recovering promptly. There was no indication that this toxicity was cumulative when the drug was given every 21 days. The effect of prior therapy on the myelosuppression suggests that patients who have received radiation to a large portion of their bone marrow space or have been treated with drugs such as mitomycin or nitrosoureas should be entered on phase II studies at a lower dose. None of the five patients treated at 170 mg/m^2 had been heavily pretreated, and the toxicity in this group was minimal. Three of the five heavily pretreated patients treated at 212 mg/m^2 had grade 4 hematologic toxicity, while only one of four patients without extensive prior therapy reached that level. The toxicity at 265 mg/m^2 was significant in all but one patient regardless of prior therapy. Based on these data, we would recommend doses of 170 and 212 mg/m^2 for heavily and minimally treated patients, respectively.

A sensory neuropathy developed in the majority of patients treated at the three highest doses. This neuropathy was very reminiscent of that seen with vinca alkaloids, with paresthesias and a glove-and-stocking distribution. In patients receiving several courses of drug, this toxicity appeared to be cumulative. Whether previous treatment with other neurotoxic drugs predisposes patients to this toxicity will require further study. Total alopecia was a consistent finding. Nausea and vomiting, mucositis, myalgias, and phlebitis occurred but were infrequent.

The serious acute toxicity which occurred with this drug in a number of studies threatened its further development. It has been assumed that these reactions were the result of the Cremophor vehicle because of similar reactions seen with other drugs formulated with it (12,13), the cardiovascular effects of Cremophor alone which were observed in animals (7), and the fact that the taxol formulation contains the highest concentration of Cremophor for a projected phase II dose (13). It is not known whether the mechanism behind this phenomenon is true anaphylaxis or whether it is nonimmunologic in character. The adverse drug reaction associated with iodinated contrast medium is one of several felt to be principally or entirely nonimmunologically mediated by the direct release of histamine and other vasoactive substances from most cells and basophils (14,15). Some chemotherapy agents also appear to stimulate the release of these substances from basophils, including perhaps the localized urticaria associated with anthracycline administration (16). The Cremophor vehicle has also been implicated in reactions attributable to histamine release in dogs (17). The fact that the reactions to taxol in both this and other studies (9) often occurred with the first dose of drug suggested that premedication based on the experience with contrast medium should be explored. The standard approach in that setting has been for pharmacologic doses of corticosteroids to be started 14–24 hours prior to drug and for an antihistamine (diphenhydramine) to be given approximately 1 hour prior (11). This had reduced the incidence of reactions in high-risk patients from 20%–35% (18,19) to approximately 5%, most of which are minor. Recently, H2 blockers such as cimetidine or ranitidine have been empirically added because blockade of both classes of receptors is necessary to fully block the cardiovascular and pulmonary effects of histamine (20). This entire regimen of premedication was adopted for use in this study, and the duration of the infusion was prolonged. The frequency and severity of acute reactions to taxol were similarly decreased, making further clinical development of this drug more realistic and worthwhile based on the antitumor activity seen. It does, however, seem prudent that initial phase II studies of taxol with this or other schedules be conducted in institutions familiar with its use, and facilities should be readily available for the management of severe type I hypersensitivity reactions.

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Phase I Study of Taxol Administered as a Short Iv Infusion Daily for 5 Days[1,2]

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Taxol inhibits cell division by promoting the assembly and stabilization of microtubules. This report describes the results of a phase I trial of taxol administered as a short iv infusion daily for 5 days every 4 weeks. Sixteen patients with refractory malignancy received 21 courses of taxol at five doses between 5 and 40 mg/m^2/day × 5. The first nine patients received taxol as a 60-minute infusion. Two patients experienced anaphylactoid reactions, one at the 5-mg/m^2/day and the second at the 15-mg/m^2/day × 5 dose levels. These reactions were characterized by facial flushing, tachypnea, and hypotension within several minutes of drug administration. These anaphylactoid reactions occurred on the first day of treatment in the first patient and on the first day of the second course in the second patient. These reactions may be related to the rapid administration of the polyoxyethylated castor oil (Cremophor EL) vehicle in which taxol is formulated. No anaphylactoid reactions were observed in the seven patients who received taxol as a 6-hour infusion with antihistamine and prednisone premedication. Dose-related myelosuppression was seen; leukopenia (wbc count < 1000/mm^3) and granulocytopenia (granulocytes ≤ 200/mm^3) occurred on Days 8 and 9 in two of two patients treated at the 40 mg/m^2/day × 5 level. Thrombocytopenia was mild, with a platelet nadir of 87,000–95,000/mm^3 at the highest dose level. Premedication with glucocorticoids and antihistamines coupled with a prolonged 6-hour infusion permitted taxol to be administered at 30 mg/m^2/day × 5 safely without immediate life-threatening reactions. [Cancer Treat Rep 71:1179–1184, 1987]

Taxol is an alkaloid derived from the Japanese yew, *Taxus brevifolia* (fig 1) (1). Taxol interacts with microtubules in intact cells as well as in cell-free systems. Treatment of microtubule preparations with taxol enhances the rate and extent of microtubule polymerization and stabilizes the microtubules, thus inhibiting depolymerization (2–5). Taxol is a potent inhibitor of cell replication, blocking cells in the G_2 and M phases of the cell cycle (6). Exponentially growing cells exposed to taxol form a mitotic apparatus that is ultrastructurally abnormal (5–7). Taxol has antineoplastic activity against experimental murine (ip B16 melanoma, P388 leukemia, and L1210 leukemia) and human (MX-1 mammary tumor in the subrenal capsule assay and the CX-1 colon and LX-1 lung xenografts) tumors (8). Taxol was active against the ip implanted P388 leukemia model; no striking schedule dependency was noted (8).

Taxol has limited solubility in water and is formulated in a vehicle containing 50% polyoxyethylated castor oil (Cremophor EL) and 50% ethanol. The formulation must be diluted in physiologic saline prior to administration. In beagle dogs, the lethal dose (LD), toxic dose high (TDH), and toxic dose low (TDL) of the clinical taxol formulation (0.6 mg of taxol per ml of vehicle) for a single iv injection were 360 mg/m^2/day × 1, 180 mg/m^2/day × 1, and 45 mg/m^2/day × 1, respectively. When taxol was administered iv on a daily × 5 days schedule, the LD, TDH, and TDL were 60 mg/m^2/day × 5, 30 mg/m^2/day × 5, and 15 mg/m^2/day × 5, respectively. Toxicity included myelosuppression, emesis, bloody diarrhea, anorexia, adipsia, weight loss, hepatic dysfunction, lymphoid depletion, hypoplasia of the germinal epithelium, and increases in total plasma lipids, cholesterol, and triglycerides (9).

Due to the need to use large infusion volumes of the clinical formulation and the sensitivity of the dog to the

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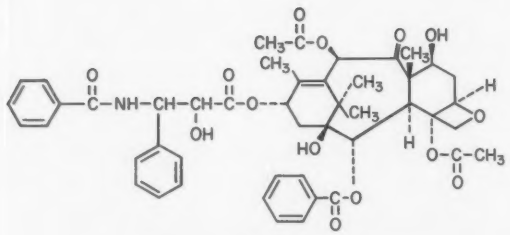

FIGURE 1.—Structure of taxol.

Cremophor component of the vehicle, studies were done in dogs to determine maximum nonlethal volumes of vehicle (9). A volume of 10 ml/kg/day × 5 of vehicle control (5% Cremophor EL, 5% ethanol, and 90% physiologic saline) administered at a rate of 2 ml/min was tolerated by the dogs, equivalent to the volume and rate of vehicle administered at the LD on the daily × 5 schedule. Larger volumes of vehicle (doses ≥ 22.5 ml/kg/day × 1) administered more rapidly (4 ml/min) were lethal to 50% of the dogs. Acute toxicity, occurring within 30–60 minutes after dosing, included vasodilation, hypothermia, labored breathing, and lethargy (9).

Because taxol has considerable antineoplastic activity against B16 melanoma and the MX-1 mammary tumor and exerts unique effects on the microtubule system, it was selected for clinical development. The current study was undertaken to determine the maximum tolerated dose (MTD) of taxol administered as a 1-hour iv infusion daily for 5 days. The starting dose for the protocol was 5 mg/m^2/day × 5 every 28 days. This starting dose was 1/12 the LD and 1/3 the TDL in dogs.

MATERIALS AND METHODS

Patient Selection

Patients with histologic evidence of advanced malignancy refractory to conventional chemotherapy or for whom no conventional therapy existed were eligible for this study. Other criteria for eligibility included Eastern Cooperative Oncology Group (ECOG) performance status of 0–3; life expectancy of ≥ 12 weeks; age ≥ 18 years; no cytotoxic therapy for at least 3 weeks prior to entering this study, with recovery from the toxic effects of such prior therapy; wbc count ≥ 4000/mm^3; granulocyte count ≥ 2000/mm^3; platelet count ≥ 100,000/mm^3; total bilirubin < 2.0 mg/dl; serum glutamic oxaloacetic transaminase (SGOT) and alkaline phosphatase (AP) < 2 × the upper limits of normal; serum creatinine < 2.0 mg/dl; blood urea nitrogen (BUN) ≤ 30 mg/dl; normal urinalysis results; no radiation therapy within 2 weeks prior to entry on study, with recovery from the toxic effects of such prior therapy; and no history of angina, myocardial infarction, stroke, or other serious cardiovascular or cerebrovascular disease. Informed consent was obtained from all patients.

Taxol Administration

Taxol was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD), as a concentrated sterile solution, 6 mg/ml in 5-ml ampoules of 50% polyoxyethylated castor oil (Cremophor EL) and 50% dehydrated alcohol, USP. The content of the ampoule was diluted in 500 ml of 0.9% sodium chloride injection, USP, to achieve a concentration of 0.015 mg/ml. In-line filtration with a 0.22-μm Acrodisc disposable filter assembly (Gelman Sciences) was used with all taxol solutions because of concern about residual particles after dilution.

The first nine patients received the calculated dose of taxol diluted in 500 ml of 0.9% saline over 60 minutes. The starting dose was 5 mg/m^2/day for 5 days, and dose escalation based on a modified Fibonacci scheme was planned. Communication with other investigators studying this agent indicated that our starting dose was well below the MTD. Therefore, we modified our dose escalation scheme and enrolled all but the first patient at doses of 15 (three patients), 20 (four patients), 30 (six patients), or 40 (two patients) mg/m^2/day for 5 days. Two of the first five patients treated developed anaphylactoid reactions during the taxol infusion (*see* Results). The next four patients were skin tested with undiluted taxol and Cremophor EL (scratch tests and intradermal tests with 0.1 ml of diluted and undiluted formulation) in an attempt to exclude patients with immediate cutaneous hypersensitivity reactions.

Since acute hypersensitivity reactions were also seen in other phase I clinical trials of taxol, the method of administration was modified to reduce the potential for immediate reactions. Seven patients received prednisone (40 mg orally at 14 hrs, 8 hrs, and 30 mins pretreatment, 8 hrs following treatment on Day 1, and then twice daily on Days 2 through 5), diphenhydramine (50 mg orally at 30 mins pretreatment and 8 hrs posttreatment on Days 1–5), and cimetidine (300 mg orally at 30 mins pretreatment and 8 hrs posttreatment on Days 1–5). The rate of infusion was slowed so that for the first hour, the infusion rate was equivalent to that required to administer the entire drug dose over 24 hours. If this was tolerated, the rate of infusion was increased to deliver the remaining drug over the next 5 hours. All infusions were administered via a mechanical infusion device to ensure the desired flow rate. A physician was in attendance for skin testing, for the initial drug infusion, and to increase the rate of infusion. Seven patients were treated with the more prolonged infusion. Courses were repeated every 28 days, providing that recovery from toxicity had occurred.

Taxol Assay

Taxol levels in plasma and urine were determined by high-performance liquid chromatography (HPLC). The instrumentation consisted of a Varian 5560 chromatograph; Rheodyne loop injector with 50-μl loop; UV-200

variable-wavelength detector set at 227 nm; and Vista 402 data system (Varian Associates, Palo Alto, CA).

For the assay of plasma samples, a 10-μm C_{18} reverse-phase column (Varian MCH-10, 4 mm × 30 cm) was used; the solvent system was 75% methanol–25% phosphoric acid (1 mM) at a flow rate of 0.75 ml/min. Plasma samples were deproteinized with 2 volumes of acetonitrile. The supernatant was diluted with an equal volume of water and applied to a prepared C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA). The cartridge was washed with 2 ml of 30% acetonitrile–water, and the taxol was eluted with 5 ml of 70% acetonitrile. The eluant was taken to dryness and reconstituted with 200 μl of the mobile phase prior to HPLC analysis. Quantitation of patient samples was achieved by comparing taxol chromatographic peak heights with those of plasma standards containing known amounts of taxol carried through the same assay procedure. Recovery of taxol from plasma averaged 91% and did not vary with taxol concentration. The standard curve was linear from 25–400 ng/ml.

Urine samples were centrifuged and diluted 1:10 with the mobile phase and then analyzed by direct injection. Quantitation was carried out by comparison with standards prepared from dilute urine spiked with known amounts of taxol. The urine standard curve was linear from 50 ng/ml to 1 μg/ml. Chromatographic conditions were altered slightly to separate taxol from endogenous urine components. A Waters C_{18} reverse-phase column was used, and the mobile phase was 58% acetonitrile–42% perchloric acid (1 mM) at a flow rate of 1.0 ml/min.

Heparinized blood samples were collected during the infusion (at 30 mins for the 1-hr infusion and at 1 and 3 hrs for the 6-hr infusion) and at the following time points postinfusion: 0, 15, 30, 45, and 60 minutes and 2, 3, 4, 6, 12, and 24 hours. Urine was collected for 2–4-hour periods during and for the first 12 hours following the infusion and then as a total 12-hour collection. Plasma and urine samples were frozen at −70°C until assayed.

RESULTS

Sixteen patients were entered in this phase I trial. The patient characteristics are shown in table 1. All patients had been previously treated with chemotherapy, and half the patients had received prior radiotherapy. Two patients developed immediate anaphylactoid reactions. The infusion was terminated immediately and was not resumed. Therefore, these two treatment cycles are evaluable only for acute toxicity. A third patient (at 15 mg/m^2/day × 5) was diagnosed as having brain metastases after receiving 1 day of therapy. Cranial radiotherapy was initiated, and no laboratory follow-up was obtained. A fourth patient (at 20 mg/m^2/day × 5) received palliative radiotherapy to a bony metastasis after only 2 days of taxol during Cycle 1; this patient received a second course of taxol after recovery from radiotherapy, which was evaluable for toxicity.

TABLE 1.—Patient characteristics*

Characteristic	
Entered	16
Female:male	7:9
Median age in yrs (range)	63 (47–77)
No. of courses	
Initiated	29
Completed and evaluable	25
Performance status (ECOG)	
0	3
1	7
2	6
Tumor type	
Colon	11
Renal	2
Lung (non-small cell)	1
Prostate	1
Adenoid cystic carcinoma of submandibular gland	1
Prior therapy	
Chemotherapy alone	8
Chemotherapy plus radiotherapy	8

*Unless stated otherwise, values = No. of patients.

Immediate Reactions

Two of the first five patients treated experienced immediate life-threatening reactions to administration of the drug. The first patient treated at the starting dose of 5 mg/m^2/day developed severe facial flushing, tachypnea, and a systolic pressure < 90 mm Hg 1 minute after the infusion was begun. The infusion was discontinued immediately; within 4 minutes, breathing had improved, facial flushing resolved, and blood pressure rose to the normal range without specific medical intervention. The patient was not rechallenged with the drug. A second patient experienced an anaphylactoid reaction on Day 1 of the second course at a dose of 15 mg/m^2/day. The patient developed nausea, dizziness, facial flushing, coughing, sneezing, and periorbital swelling within 1 minute of the infusion. The patient lost consciousness for 2 minutes, the systolic blood pressure fell to 40 mm Hg, and respiration decreased to 8–10 breaths per minute. The drug was immediately discontinued, and normal saline was administered iv. The blood pressure promptly rose to 105 mm Hg systolic. Upon regaining consciousness, he vomited three times. An electrocardiographic recording revealed a rate of 104 beats per minute and a bigeminal rhythm. He was monitored in an intensive care unit with a normal heart rate and rhythm and blood pressure ≥ 120 mm Hg systolic. No residual toxicity was noted, and he was discharged 3 days later in good condition. This patient was not retreated.

The next four patients had intradermal and scratch tests with 0.1 ml of the diluted and undiluted formulation of taxol. No immediate-type cutaneous hypersensitivity reactions were seen. These patients subsequently received their appropriate dose as a 60-minute infusion without difficulty. The two patients who did experience

anaphylactoid reactions during taxol administration were skin tested 1 and 3 months after taxol exposure; both patients had negative results.

After the rate of administration was slowed and premedication with prednisone, diphenhydramine, and cimetidine was instituted as outlined in Materials and Methods, no immediate anaphylactoid reactions were observed in seven patients. One patient developed a transient pruritic rash on the arm receiving the iv infusion on Day 3 of the second course. The erythema and itching cleared within 30 minutes after discontinuing the infusion. Skin testing was not performed for the remaining seven patients because the emerging clinical data suggested that the immediate toxic reactions were related to the rate of administration of taxol.

Hematologic Toxicity

Myelosuppression was dose limiting (table 2). Minimal to mild myelosuppression was observed at 15 and 20 mg/m^2/day × 5. At 30 mg/m^2/day × 5, one of six patients developed a wbc count < 2000 cells/mm^3 and granulocytopenia (< 1000 cells/mm^3). In neither patient did the platelet count fall to < 100,000/mm^3. This patient had previously received pelvic irradiation followed by iv 5-fluorouracil. At 40 mg/m^2/day × 5, two of two patients experienced severe leukopenia with absolute wbc counts of 600 and 900 cells/mm^3 and granulocyte counts of 100 and 200 cells/mm^3, respectively. The nadir wbc count at 40 mg/m^2 occurred on Days 8 and 9, with recovery by Days 14 and 22. The nadir platelet counts at the full dose were 87,000/mm^3 and 95,000/mm^3. One patient received an additional three courses at 20 mg/m^2/day with a nadir wbc count 2000–2999 cells/mm^3 following each of these three courses. One patient treated at 40 mg/m^2 had received only acivicin; the other had undergone pelvic radiation therapy and received 5-fluorouracil.

Other Toxic Effects

Mild to moderate nausea and vomiting, controllable by antiemetic therapy, occurred in three patients (19%) on the days of treatment (*see* ref 12 for ECOG toxicity criteria) and only occurred at the 30-mg/m^2/day × 5 level. Diarrhea did not occur. Ulceration of the oral mucosa occurred in one patient (6%), and two patients complained of a sore throat (12%); all three patients received ≥ 30 mg/m^2/day × 5. Moderate alopecia occurred in one patient at 30 mg/m^2/day × 5. Neurologic toxicity was not observed. Six patients (38%) experienced mild to severe fatigue lasting up to 7 days after the completion of taxol administration. Five of the six patients who complained of fatigue were treated at 30–40 mg/m^2/day and received a 5-day course of prednisone in the hospital. One of these five patients did not receive prednisone during his fourth course of taxol, and fatigue did not recur. Therefore, it is unclear whether the fatigue was related to taxol dose, volume of vehicle, the short course of high-dose prednisone followed by steroid withdrawal, or a combination of factors. Elevations of liver enzymes occurred in two patients (12%) treated at 30 mg/m^2/day. One woman experienced a rise in SGOT to 540 U/L (normal, 0–40) on Day 21, accompanied by a rise in LDH to greater than 2 × normal and a rise in bilirubin to 1.1 mg/dl, compared with her baseline of 0.4 mg/dl (normal, 0.5–1.4). A computerized tomographic scan of the liver was normal. The SGOT returned to the normal range 4 weeks later. Another patient with known hepatic metastases from colon cancer developed a rise in bilirubin and AP to 3.0 mg/dl and 351 U/L, respectively, on Day 11 of Course 1. The bilirubin returned to the normal range by Day 28, although AP remained > 2 × normal. Neither patient was retreated with taxol. Three patients at the 30-mg/m^2/day × 5 level experienced mild elevations in BUN (30–40-mg/dl range) without a concomitant change in serum creatinine. Two patients experienced infections; one developed peritonsillar cellulitis while neutropenic, and another patient developed oral candidiasis.

Response

No complete or partial responses were observed in six patients with measurable disease or in eight patients with evaluable disease.

Pharmacokinetics

Plasma taxol was measured by the HPLC procedure outlined above. Due to the small amount of taxol administered in a single dose on the daily × 5 schedule and the prolonged infusion time used at the higher doses, the peak taxol levels were relatively low and declined rapidly to levels below the sensitivity of the assay. Since

TABLE 2.—Hematologic toxicity

Dose (mg/m^2/day × 5)	No. of patients (n = 16)	No. of evaluable courses (n = 25)	Median wbc count nadir, cells × 10^3/mm^3 (range)	Median day of nadir (range)	Median platelet count nadir, cells × 10^3/mm^3 (range)	Median granulocyte count nadir, cells × 10^3/mm^3 (range)	Median day of nadir (range)
5	1	0					
15	3	7	7.6 (5.1–9.4)	15 (5–16)	383 (113–643)	6.7 (3.9–8.1)	15 (5–29)
20	4	8	7.15 (2.6–9.6)	15 (15–19)	310 (181–669)	4.0 (1.7–8.1)	15 (11–15)
30	6	8	5.1 (1.1–12.0)	16.5 (8–29)	390 (257–562)	3.65 (0.4–10.8)	15 (8–29)
40	2	2	0.75 (0.6–0.9)	8.5 (8–9)	91 (87–95)	0.15 (0.1–0.2)	11

TABLE 3.—Taxol pharmacokinetic parameters*

Patient No.	Dose (mg/m^2)	Infusion time (min)	C_p (ng/ml)	AUC (min·mg/L)	$t_{½}$ (min)	Cl_{TB} ($L/hr/m^2$)	eV_β (L/m^2)
1	40	360	248	47	62	51	76
2	30	360	168	53	36	34	29
3	30	360	54	16	54	110	143
4	30	360	191	96	172	19	79
Mean ± SD					81 ± 53	54 ± 40	82 ± 40
5	30	60	297	22	56	81	109
6	20	60	291	25	131	48	53
7	15	60	238	16	64	55	85
8	15	60	312	34	115	27	75
Mean ± SD					92 ± 32	53 ± 19	80 ± 20
Overall mean ± SD					86 ± 44	53 ± 28	81 ± 32

*C_p = peak plasma taxol concentration; AUC = area under the concentration-time curve; $t_{½}$ = terminal half-life; Cl_{TB} = taxol total body clearance (dose/AUC); and eV_β = apparent volume of distribution (area) (= Cl_{TB}/terminal rate constant).

taxol was only detectable for 2–4 hours postinfusion, too few data points were available for good curve-fitting, and detailed analysis of taxol pharmacokinetics was not possible. The plasma concentration-time data from the start of the infusion were analyzed by standard non-compartmental methods (10,11). Total area under the curve (AUC) was determined by the linear trapezoidal rule extrapolated by C_{last}/β. The terminal rate constant β was determined by log-linear least-squares fit of the terminal portion of the curve. Pharmacokinetic parameters are presented in table 3. Total plasma clearance was variable, ranging from 20–> 100 $L/hr/m^2$ (mean, 53). It is interesting to note that changing the infusion time did not apparently alter the taxol total body clearance (54 $L/hr/m^2$ on the 6-hour schedule and 53 $L/hr/m^2$ on the 1-hour schedule). The volume of distribution averaged 81 L/m^2. The mean terminal half-life was found to be about 1.5 hours. Complete 24-hour urine collections were obtained from only two patients. A mean of 6.6% of the total dose was recovered as intact taxol in these samples.

DISCUSSION

The most striking toxicity seen in this trial was a severe anaphylactoid reaction consisting of shortness of breath, hypotension, facial edema, and skin redness. These reactions occurred within 1 minute of initiating the infusion during the initial exposure in the first patient and on the first day of the second course in the second patient. Similar reactions have been noted in other clinical trials employing single iv short infusions at doses of 25–175 $mg/m^2 \times 1$ (13).

Cremophor EL is used as a solubilizing agent for several drugs, including teniposide, echinomycin, and didemnin B, the anesthetic agents althesin and propanidid, the fat-soluble vitamins A, D, E, and K, and cyclosporine (13–15). In England, severe anaphylactoid reactions have been reported following the injection of propanidid and althesin with an incidence of 1:1000–1:7000 applications (16–18). Reactions consisting of shortness of breath, hypotension, flushing, and bronchospasm occur in 3%–10% of patients who receive teniposide, echinomycin, or didemnin B (13–15). These immediate reactions to teniposide did not appear to be dose related and often occurred on the first exposure to the drug. Rapid iv administration of teniposide appears to be more often associated with hypotensive reactions than slower infusions (15). Cremophor EL causes histamine release in dogs and cats (16,19). Lorenz et al (16) found that the oxyethylated oleic acid constituent (accounting for about 50% of Cremophor EL) released histamine and produced hypotension in dogs (16). In the preclinical toxicology studies of taxol in beagle dogs, vehicle control doses of 22.5, 30, and 40 ml/kg administered at 4 ml/minute produced cardiovascular collapse in 50% of the dogs. Histamine levels were not measured in these dogs (9). A vehicle volume of 10 ml/kg daily × 5 and a single dose of 20 ml/kg administered at 2 ml/minute were tolerated, suggesting that the speed of injection, initial drug concentration, or a combination of these factors might be important in triggering the hypotensive response. The amounts of the Cremophor EL-ethanol components of the vehicle infused per minute in our two patients who experienced anaphylactoid reactions were approximately one-tenth and one-third of that associated with cardiovascular collapse in the dogs. In clinical trials in which taxol was administered as a continuous infusion over 24 hours, only one immediate reaction consisting of hypotension and shortness of breath was observed among 23 patients who were treated with this schedule. In contrast, 12 anaphylactoid reactions were noted in 79 patients receiving taxol as an infusion over 1–4 hours.[7]

These immediate reactions in our patients and others reported previously who have received chemotherapy drugs formulated in Cremophor EL are reminiscent of radiographic contrast medium reactions. Anaphylactoid

[7] Personal communication, M. Lassus, Investigational Drug Branch, Cancer Treatment Evaluation Program, Division of Cancer Treatment, National Cancer Institute.

reactions to such iodinated media mimic IgE-mediated hypersensitivity in that the reaction is immediate and may include urticaria, angioedema, generalized pruritis, bronchospasm, hypotension, or shock (20). Although the pathogenesis of reactions to contrast dye has not been precisely defined, radiocontrast medium is thought to be capable of direct mast cell degranulation, resulting in release of biologically active chemical mediators such as histamine (20,21). Premedication regimens containing glucocorticoids and diphenhydramine have reduced the reaction rate in patients with a history of anaphylactoid reactions to contrast medium (20). Premedication with glucocorticoid and antihistamines coupled with slowing the rate of taxol infusion to 6 hours were instituted for the last seven patients entered at 30–40 mg/m^2/day. One patient did experience a transient skin rash during the longer infusion schedule, but no anaphylactoid reactions were seen.

The mean peak plasma level reached at a dose of 30 mg/m^2/day with a 6-hour infusion was 0.14 μM (122 ng/ml). This concentration is substantially lower than the minimal levels (0.25–1.0 μM) shown in vitro to affect the cell cycle and microtubule polymerization (2,6). The large volume of distribution, high but variable total body clearance, and low renal clearance values are consistent with those recently reported by other investigators (22,23). However, due to the short period of time over which we were able to measure the decline in plasma taxol, the terminal half-life reported here is shorter, and we should also note that the clearance may be somewhat overestimated.

The dose-limiting toxicity for taxol on the daily × 5 6-hour infusion schedule was leukopenia. The anaphylactoid reactions appear to be related to rapid infusion of the vehicle and are not clearly dose-related. Although pharmacologic release of histamine may be operative in humans, complement activation, antibody-dependent responses, or other mechanisms could also be contributory in some instances. These findings are similar to the clinical data of other investigators (24,25). Taxol at 30 mg/m^2/day × 5 is a safe dose. The requirement for prolonged infusion and lack of striking schedule dependency for taxol in preclinical models led to a decision to develop schedules of taxol other than the daily × 5. Consequently, precise definition of a phase II starting dose on this schedule was not pursued. There was little rationale for treating patients at doses between 30 and 40 mg/m^2 simply to define a starting dose.

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Cisplatin, Vinblastine, and Mitoguazone in Squamous Cell Carcinoma of the Esophagus: A Southwest Oncology Group Study[1,2]

Robert Chapman,[3] Thomas R. Fleming,[4] John Van Damme,[4] and John Macdonald[5,*]

Forty patients with locally extensive or metastatic squamous cell carcinoma of the esophagus were registered onto a phase II trial employing cisplatin, vinblastine, and mitoguazone. Of 36 eligible patients, four (11%) had partial responses. None had received prior chemotherapy or radiation therapy. Toxicity was mild to moderate and consisted mostly of nausea and vomiting. The activity of this regimen at the doses and schedule used was minimal. More aggressive use of therapy should be considered for further trials. [Cancer Treat Rep 71:1185-1187, 1987]

Squamous cell carcinoma of the esophagus (SCCE) remains a vexing problem. Recent combined-modality approaches have yielded some improvement in local disease control, but overall survival remains poor (1). Clearly, improved local and systemic therapy is necessary to improve the outlook in this disease.

Recently, cisplatin and cisplatin-containing combinations have shown objective activity in advanced esophageal carcinoma (2-4). Mitoguazone hydrochloride (MGBG) has shown minimal activity as a single agent in this disease (5), but its lack of myelosuppression as a toxic effect makes it an attractive drug to use in combination with other agents. In an effort to identify therapy with useful systemic activity, the Southwest Oncology Group (SWOG) initiated a phase II trial of cisplatin, MGBG, and vinblastine combination chemotherapy in patients with advanced unresectable SCCE who had had no prior therapy.

MATERIALS AND METHODS

Forty patients with locally extensive and metastatic SCCE were entered onto this trial between August 1983 and March 1985. All patients had objectively measurable metastatic disease or evaluable tumor by barium esophogram. Histologically proven epidermoid carcinoma of the esophagus was required. Eligible patients were required to have adequate renal (creatinine clearance $\geq$ 60 ml/minute) and hepatic (serum bilirubin $\leq$ 3.0 mg/dl) function, as well as a wbc count $\geq$ 4000/μl and a platelet count $\geq$ 100,000/μl. All patients were required to have a fasting blood sugar $\geq$ 60 mg/dl, and patients with symptomatic congestive heart failure were ineligible. No patients had prior radiation therapy or chemotherapy. Informed consent was obtained from each patient.

Patients were registered through the SWOG operations office and stratified as having either metastatic or locally extensive disease. Locally extensive disease was defined as being clearly unresectable by virtue of direct extension into either the mediastinum or adjacent parenchymal structures.

MGBG was supplied by the Investigational Drug Branch of the National Cancer Institute. MGBG was administered at 400 mg/m^2 over 1-1.5 hours every 2 weeks. Vinblastine was given at a dose of 2 mg/m^2 on Days 1, 2, 15, 16, 29, and 30 and then on 2 consecutive days every 3 weeks. Cisplatin (90 mg/m^2) was administered on Days 1 and 29 and every 6 weeks thereafter with saline prehydration and mannitol-induced diuresis. The choice of antiemetic therapy was left to the discretion of the individual investigator.

Patients with locally extensive disease were reevaluated for resectability after 6 weeks. Clinically operable patients were eligible for resection; otherwise they were to receive radiation therapy. Patients with metastatic disease were treated until they developed progressive disease or had intolerable toxicity.

The dose of vinblastine in subsequent cycles was decreased 25% for a wbc count of 2500-3000/μl or serum bilirubin 1.5-2.5 mg/dl. Vinblastine was reduced 50% for bilirubin 2.5-3.0 mg/dl and withheld if the bilirubin level was higher. Vinblastine and cisplatin were withheld until recovery for a wbc count $<$ 2500/μl or platelet

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TABLE 1.—Patient characteristics*

Characteristic	
Median age in yrs (range)	61 (45–81)
Median Zubrod performance status (range)	2 (0–3)
Extent of disease	
Locally extensive	12 (33)
Metastatic	24 (67)
Sex	
Male	30 (83)
Female	6 (17)
Race	
Black	23 (64)
White	12 (33)
Other	1 (3)

*Unless stated otherwise, values = No. of patients (%).

count $< 50{,}000/\mu l$. Cisplatin was reduced to 60 mg/m^2 on subsequent cycles for creatinine clearance of 50–60 ml/minute and withheld if creatinine clearance was lower.

Response criteria were as follows: *complete response*—the complete disappearance of all clinical evidence of existing disease; *partial response*—disappearance of dysphagia and a $\geq 50\%$ reduction in the sum of the products of perpendicular measurements of measurable lesions or a major reduction in size of index lesions (including esophograms) of evaluable lesions; *stable disease*—a $< 50\%$ regression or $< 25\%$ increase in measurable lesions; *progressive disease*—a $\geq 25\%$ increase in size of any measurable lesion, appearance of any new lesion, or worsening of an evaluable clinical parameter. Response duration was measured from the time at which a response was achieved until the time that disease progression was documented.

RESULTS

Of the 40 patients registered onto the trial, 36 were eligible. Reasons for ineligibility were no measurable disease, unacceptable serum creatinine level at registration, no locally extensive or metastatic disease, and failure to obtain baseline tumor measurements. Patient characteristics are summarized in table 1. All eligible patients were included in the evaluation for response and toxicity. Three patients had early, non-treatment-related deaths occurring at Days 9, 20, and 29 of protocol therapy. Two patients requested cessation of treatment after 1 and 5 months on therapy. Two patients were removed from study by the treating investigator because of an unrelated chronic infection and unacceptable constitutional symptoms, respectively.

There were no complete responders. Four patients had documented partial responses lasting 6, 12, 14, and 24 weeks. Nine patients had stable disease, and the others had increasing disease. Overall median length of survival from registration was 3.4 months.

Toxicity

Toxicity, outlined in table 2, was no more than moderate in the majority of patients. Three patients did have life-threatening toxicity due to hypotension, thrombocytopenia, and leukopenia. There were no treatment-related fatalities. Mild to moderate nausea and vomiting was by far the most common toxic effect.

DISCUSSION

This trial, employing combination chemotherapy in advanced SCCE, produced an 11% response rate with no durable responses. This result is considerably poorer than that reported by Kelsen et al (3,4) for similar therapy. There may be several reasons that at least partially explain the difference. Recently, a direct dose-response relationship was suggested for a regimen including cisplatin, vinblastine, and MGBG in a phase I–II trial in non-small cell bronchogenic carcinoma (6). The relatively mild toxicity in this study suggests that future trials should use more aggressive therapy. In addition, the majority of the patients in this trial had metastatic disease, and most had a poor performance status.

These results underscore the fact that advanced SCCE remains a very difficult therapeutic challenge for the clinician and the clinical investigator. Clearly, well-conceived aggressive trials in the future are needed.

TABLE 2.—Toxic effects*

Toxic effect	No. of patients ($n = 36$) with toxicity of grade—				
	0	1	2	3	4
Alopecia	35	1	0	0	0
Anemia	34	0	0	2	0
Cardiac hypotension	34	1	0	0	1
Leukopenia	27	2	4	2	1
Mucositis	35	1	0	0	0
Nausea/vomiting	11	12	8	5	0
Thrombocytopenia	34	0	0	0	2
Uremia	30	1	3	2	0
Maximum degree of any toxic effect	9	7	8	9	3

*Toxicity was defined by a standard scale utilized by cooperative groups (7).

Although combined-modality therapy has shown some promise in patients with regionally confined disease (1), such approaches offer little hope to date for the patient with metastatic SCCE.

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In Vitro Stability and Compatibility of Daunorubicin, Cytarabine, and Etoposide[1,2]

Lorne E. Seargeant, Nathan L. Kobrinsky,* Catherine J. Sus, and Don R. Nazeravich[3,4]

The continuous infusion of cytarabine, daunorubicin, and etoposide offers several theoretical advantages over bolus infusion in the treatment of acute nonlymphocytic leukemia. To date, this approach has been limited by the need for three separate iv lines. The in vitro stability and compatibility of these three agents were therefore evaluated. Solutions of 200 mg of cytarabine, 25 mg of daunorubicin, and 300 mg of etoposide per 750 ml of 5% dextrose and 0.45% saline were prepared alone and in combination. The solutions were evaluated visually, spectrophotometrically, and by high-pressure liquid chromatography (HPLC) twice daily for 72 hours. Precipitates or color changes were not noted. Changes in the patterns of the spectral scans and chromatographs were not observed. Concentrations of the drugs as assessed by HPLC were stable over the 72-hour period of observation for both individual and combined drug preparations. In conclusion, cytarabine, daunorubicin, and etoposide are stable and compatible in vitro for at least 72 hours. These drugs can therefore be administered together by continuous infusion using a single iv line. [Cancer Treat Rep 71:1189-1192, 1987]

Cytarabine, daunorubicin, and etoposide used individually or in combination are effective drugs in the treatment of acute nonlymphocytic leukemia (1-4). Although various methods of administration have been used, cytarabine has in general been administered by continuous infusion and daunorubicin and etoposide have been administered by bolus infusion. Recent data suggest that continuous infusion may be a preferred method of administration for all three drugs. Cardiac toxicity can be markedly decreased without loss of efficacy when anthracyclines are administered by continuous infusion (5-7). Hematologic and gastrointestinal toxicity may be decreased when etoposide is administered once every 12 hours for six doses rather than once every 24 hours for three doses, suggesting a theoretical benefit for continuous infusion (8,9). A phase I trial of continuous infusion etoposide has been reported (10).

Administration of all three drugs by continuous infusion, however, raises logistical problems. The compatibility of these agents has not previously been reported, and accordingly separate iv lines would therefore be required. In a patient undergoing induction therapy, this poses several problems. The risks of infection and thrombophlebitis are greatly increased. The requirement for repeated venipunctures is accordingly increased. Thus, the venous access necessary to maintain three lines with probable requirements for other medications would make this a completely impractical consideration.

The present study was therefore undertaken to determine the in vitro stability and compatibility of cytarabine, daunorubicin, and etoposide.

MATERIALS AND METHODS

Preparation of Solutions

Cytarabine (Upjohn, Canada), daunorubicin (Rhone Poulenc, Canada), and etoposide (Bristol, Canada) were reconstituted and added individually and together to polyvinyl chloride bags containing 5% dextrose and 0.45% sodium chloride. The final concentrations of the individual drugs were 200 mg of cytarabine, 25 mg of daunorubicin, and 300 mg of etoposide per 750 ml of 5% dextrose and 0.45% sodium chloride.

All solutions were prepared immediately prior to testing and evaluated at 0, 8, 24, 32, 48, 56, and 72 hours by

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[4] We thank Dr. William Woods of the University of Minnesota Teaching Hospitals, Dr. Beverley Lange of the Children's Hospital of Philadelphia, and Dr. Jack Priest of the St. Paul Children's Hospital for providing clinical information concerning the patients treated with the continuous-infusion regimen described in this report.
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(*a*) visual inspection, (*b*) spectrophotometric analysis, and (*c*) high-pressure liquid chromatography (HPLC). The solutions were stored at room temperature (20°C) and not protected from light.

Spectrophotometric Analysis

A 1-ml aliquot was removed from each iv bag and diluted 20 times with water. The ultraviolet spectrum was then obtained from 225 nm to 310 nm. Spectral scans were plotted with a Bausch and Lomb Spectronic 2000 spectrometer (Bausch and Lomb Inc, Rochester, NY).

High-Pressure Liquid Chromatography

A 1-ml aliquot was removed from each iv bag. An internal standard, 10 μg of p-toluic acid (Sigma), was then added, and the aliquot was filtered (0.22-μm filter; Fisher Scientific). A 50-μl sample was then injected into the chromatograph.

HPLC was performed at ambient temperature on a Varian 8500 HPLC system equipped for gradient analysis (Varian Associates, Sunnyvale, CA). The system included a Varian C_{18} reverse-phase column (MCH-10 Micropak, 30 cm × 4 mm), Waters U6K injector (Waters Associates, Milford, MA), and Varian Varichrom UV detector set at 235 nm. Peak areas were measured on a Hewlett Packard 3390A integrator (Hewlett Packard, Avondale, PA). Buffer A contained sodium phosphate buffer (10 mmol/L) and 3% (v/v) acetonitrile (Baker, HPLC grade). Buffer B was 100% acetonitrile. Drugs were eluted with a gradient from 100% buffer A–0% buffer B to 45% buffer A–55% buffer B. Standard curves were plotted by using either peak area or the ratio of standard area to the p-toluic acid area (internal standard).

RESULTS

Visual Inspection

The individual and combined solutions of cytarabine, daunorubicin, and etoposide did not change over the 72-hour period of observation. Precipitates and color changes were not observed.

Spectrophotometry

The spectral scans of the three drugs in solution obtained immediately after mixing (A) and after 72 hours of incubation (B) are illustrated in figure 1. For both individual and combined solutions, changes in the spectral scan patterns were not observed.

High-Pressure Liquid Chromatography

The chromatographs of the three drugs in solution obtained immediately after mixing (A) and after 72 hours of incubation (B) are illustrated in figure 2. For both individual and combined solutions, the components were well resolved, and changes in the chromatographic patterns were not observed. All minor components detected in the combined solution coincided with components in the individual drug solutions. The peak areas for each of the three drugs alone and in combination were unchanged for the 72-hour period of observation. The peak areas for the combined solution are expressed as percentages of the peak areas in the individual solutions in table 1.

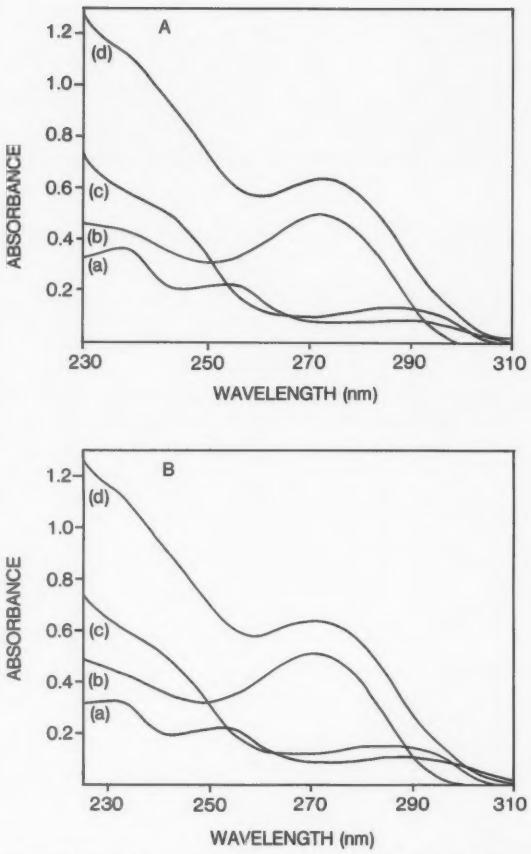

FIGURE 1.—Spectral scans of (a) daunorubicin, (b) cytarabine, (c) etoposide, and (d) all three drugs together at (A) Hour 0 and (B) Hour 72.

DISCUSSION

The present study demonstrates that cytarabine, daunorubicin, and etoposide are stable and compatible in vitro for at least 72 hours at room temperature. The drug concentrations used were based on a theoretical dose schedule of 200 mg/m^2 of cytarabine, 25 mg/m^2 of daunorubicin, and 300 mg/m^2 of etoposide in a fluid volume of 750 ml/m^2/day. This volume represents approximately 25% of the daily fluids administered during induction and therefore should not limit the fluid volume available for other iv drugs. Although more

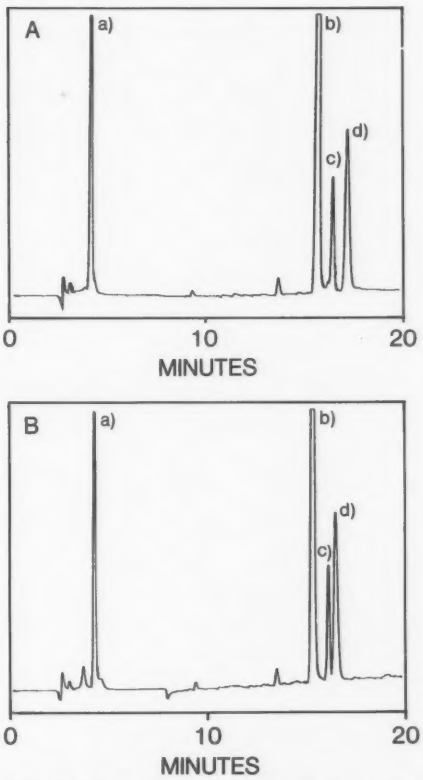

FIGURE 2.—HPLC of (a) cytarabine, (b) etoposide, (c) the internal standard (*p*-toluic acid), and (d) daunorubicin at (A) Hour 0 and (B) Hour 72. Retention times were similar for drugs in the combined and individual solutions.

concentrated solutions of cytarabine and daunorubicin may have been stable, the concentration of etoposide (0.4 mg/ml) used was the maximum concentration recommended by the manufacturer.

Cytarabine administered by continuous infusion and daunorubicin administered by bolus infusion have been used for many years in the treatment of acute nonlymphocytic leukemia (1,2). Recent studies have demonstrated a marked decrease in cardiac toxicity when anthracyclines are administered by continuous infusion (5–7). Despite these observations, daunorubicin is still administered by bolus infusion, primarily because of the impracticality of maintaining two continuous-infusion lines. The present study demonstrates that both agents can be administered by using a single iv line.

TABLE 1.—Peak areas of drug in combined solution as percentage of peak area in individual solutions*

	% of individual peak area in combined solution		
Time (hrs)	Cytarabine	Daunorubicin	Etoposide
0	102	106	99
24	116	109	98
48	97	109	100
72	94	107	100

*The peak areas for the internal standard (p-toluic acid) were within the analytical variation expected for the analysis (coefficient of variation, < 5%).

Etoposide administered as a single agent has been shown to be effective in the treatment of acute nonlymphocytic leukemia. Cytokinetic studies have consistently demonstrated G_2 arrest of malignant myeloblasts (11). In 1979, the Children's Cancer Study Group reported complete responses in two of 44 patients with acute myeloblastic leukemia and in three of 18 patients with acute myelomonocytic leukemia receiving etoposide as a single agent (12). Similar responses in other pediatric and adult patient series have been reported (3,13). Etoposide has been particularly effective when combined with 5-azacitidine or cyclophosphamide (4,14). Based on these observations, etoposide would appear to be a particularly promising agent to be used in combination with cytarabine and daunorubicin. The present study demonstrates that all three agents can be administered by continuous infusion with a single iv line.

A preliminary clinical trial using "single-bag" continuous-infusion cytarabine, daunorubicin, and etoposide in the dosage schedule described in this report daily for 4 days is ongoing at present. Twenty-four-hour-infusion bags prepared under laminar-flow conditions were used to ensure microbial sterility. Unexpected side effects have not been observed either during the infusion or during the subsequent period of recovery in the first 20 courses administered. Although the compatibility and stability of this combination have been demonstrated in vitro and in the clinical setting, therapeutic efficacy remains to be established.

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Phase II Study of Iproplatin in Metastatic Breast Carcinoma[1,2,3]

G. N. Hortobagyi,* D. Frye, F. A. Holmes, V. Hug, G. Fraschini, and A. U. Buzdar[4]

Thirty patients with progressive metastatic breast cancer and one prior chemotherapy regimen were treated with iproplatin at a starting dose of 300 mg/m² iv every 3 weeks. After the first 11 patients, the starting dose was decreased to 270 mg/m². There were one complete remission, three partial remissions, and two minor responses. Responses were observed in soft tissue and osseous and visceral areas. Grade 3 nausea and vomiting were observed in 38% of patients, and grade 3 diarrhea occurred in 31%. The dose-limiting toxicity was thrombocytopenia, which required dose de-escalation in 15 patients. No nephrotoxicity, neurotoxicity, or ototoxicity was observed. Iproplatin has modest antitumor activity in this group of previously treated patients with metastatic breast cancer. [Cancer Treat Rep 71:1193-1196, 1987]

Cisplatin is an active antitumor agent against metastatic breast cancer (1-3). However, nephrotoxicity and neurotoxicity limit its long-term use. *cis*-Dichloro-*trans*-dihydroxy-bis(isopropalamine)-platinum(IV) (iproplatin; CHIP) is a new, second-generation platinum analog which has demonstrated antitumor activity in experimental animals comparable to that of cisplatin, but with less nephrotoxicity and neurotoxicity. It is active against L1210 leukemia, B16 melanoma, Lewis lung carcinoma, Walker carcinosarcoma 256, and the Murphy-Sturm lymphosarcoma (4). Several phase I studies have been completed (5-7). The maximum tolerated dose was found to be between 250 and 350 mg/m² when the drug was administered every 3-4 weeks, or 95 mg/m²/week. The dose-limiting toxicity with either schedule was thrombocytopenia, but nausea, vomiting, diarrhea, stomatitis, and a rash were also observed. Antitumor activity against several solid tumors was observed during the phase I trials. Based on these results, we evaluated iproplatin in a population of patients with metastatic breast cancer.

PATIENTS AND METHODS

Patients with histologic proof of breast cancer and evidence of measurable metastatic lesions were eligible for this study. They were required to have a performance status < 3, no antitumor therapy for the 3 weeks prior to starting treatment with iproplatin, and adequate bone marrow (absolute granulocyte count $\geq 1800/mm^3$, platelet count $> 100,000/mm^3$), hepatic (bilirubin ≤ 2.0 mg/dl), and renal function (serum creatinine < 1.2 mg/dl). Prior cisplatin therapy, more than one prior chemotherapeutic regimen, the presence of CNS metastases, or a history of another malignancy excluded patients from this clinical trial. Written informed consent was obtained from each patient. Iproplatin was administered at a starting dose of 300 mg/m² dissolved in 100 ml of normal saline, as a 15-30-minute iv infusion every 28 days. Because of severe thrombocytopenia among the first 11 patients, the starting dose was reduced to 270 mg/m². If the lowest recorded platelet count was $\leq 50,000/mm^3$ or the lowest recorded granulocyte count was $\leq 500/mm^3$, the dose was reduced by 20%. If the lowest recorded platelet count was $> 75,000/mm^3$ and the lowest recorded granulocyte count was $> 1000/mm^3$, a 10% increase in dose was required. Prior to initiation of therapy, a complete history (including performance status, recent weight loss, and prior therapy) and physical examination were done. Laboratory studies included a cbc, a biochemical survey (SMA-12), carcinoembryonic antigen, urinalysis, and electrolytes; in addition, a chest x-ray and appropriate imaging studies to fully define the extent and severity of malignant disease were obtained. During the study, a weekly cbc with differential and platelet counts was obtained; prior to each treatment, the biochemical survey, including renal and hepatic function tests, was done. Tumor measurements

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[3] Presented in part at the 78th Annual Meeting of the American Association for Cancer Research, Atlanta, GA, Mar 1987.
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were recorded before each course, taking the longest diameter and its perpendicular in centimeters. All patients were registered prospectively with a central data management office.

An estimate of overall objective and subjective response was made at each visit. Response to therapy was evaluated by standard criteria (8). Measurable disease and response to therapy were scrutinized by several investigators, and response data represent the consensus of a group of three, including the principal investigator. Response duration was measured from the time of response; time to progression and survival were measured from the initiation of therapy.

RESULTS

Thirty patients were registered on this study between September 1985 and November 1986. Their pretreatment patient characteristics are described in table 1. Two patients had received two prior chemotherapy regimens. However, in both instances the regimen used for adjuvant chemotherapy was used again for treatment of metastatic disease, so the two prior regimens were the same. Most patients had an excellent performance status and had one or two organ sites of metastatic involvement. One patient died of nonneutropenic pneumonia 2 weeks after the initiation of therapy and without evaluation of her tumor for response. The other 29 patients were evaluable for response. There were four major (one complete and three partial) objective remissions (13.3%). The complete remission occurred in a large supraclavicular node, while partial remissions were observed in soft tissue lesions, lung, mediastinum, liver, bone, and pleural effusion. There were two minor responses. One patient achieved a > 50% decrease in a very large locally advanced breast cancer, but died of lactic acidosis before 1 month in partial remission was completed. Six patients had no change, and the remaining 17 had progressive disease. Partial responses lasted 4, 5, and 7 months, and the complete response continues at 5 months. The two minor responses lasted < 1 month and 3 months. Median survival has not been reached but will exceed 7 months. Some evidence of response was obtained in all responding patients after the first cycle, and for three of the four patients with a major response this evidence was already a partial remission after the first cycle. The complete remission was obtained after five cycles of therapy.

Toxicity is summarized in table 2. Grade 3 nausea and vomiting were observed in 38% of patients despite routine use of antiemetics. Grade 3 diarrhea was reported by nine patients. In some of them, premedication with diphenoxylate decreased the severity of this side effect. A maculopapular skin rash which was intensely pruritic and/or painful was seen in four patients but was minor in two of them. The two patients who had extensive skin rash had this toxic effect develop at a time of severe thrombocytopenia, and the rash was

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Entered	30
Median age in yrs (range): 55 (31-71)	
Menopausal status	
Premenopausal	4 (13)
Postmenopausal	26 (87)
Estrogen receptor status	
Positive (> 10 fmoles/mg)	16 (53)
Negative	9 (30)
Unknown	5 (17)
Performance status	
0	8 (26.7)
1	10 (33.3)
2	11 (36.7)
3	1 (3.3)
No. of metastatic sites	
1	14 (47)
2	9 (30)
3	5 (17)
> 3	2 (7)
Dominant site of disease	
Soft tissue	7 (23)
Bone	8 (27)
Visceral	15 (50)
Prior therapies	
Chemotherapy	30 (100)
Adjuvant	19 (63)
For metastases	11 (37)
Hormones	21 (70)
Immunotherapy	3 (10)
Radiotherapy	20 (66.7)
No. of prior chemotherapy agents	
2	2 (6.6)
3	13 (43.3)
4	10 (26.7)
> 4	5 (23.3)

TABLE 2.—Toxicity*

Effect	Grade	No. of patients (%)
Nausea/vomiting	0	1 (3)
	1	4 (13.8)
	2	13 (45)
	3	11 (38)
Diarrhea	0	7 (24)
	1	5 (17)
	2	8 (28)
	3	9 (31)
Malaise	0	19 (65)
	1	1 (3)
	2	5 (17)
	3	4 (14)
Skin rash	0	25 (86)
	1	2 (7)
	2	0
	3	2 (7)
Petechiae/mild bruising	0	21 (72)
	1	8 (28)

*Data for 1 patient treated mistakenly at 400 mg/m^2 are not included in this table but are described in the text.

TABLE 3.—Hematologic effects of iproplatin, all courses

Dose level	No. of patients evaluable	No. of courses	Median granulocyte count nadir, 10^3 cells/ml (range)	Median Day of nadir	Median platelet count nadir, 10^3 cells/ml (range)	Median Day of nadir
−5	1	1	1.6 (1.6–1.6)	16	245 (245–245)	26
−4	5	8	1.4 (0.7–3.4)	16	60 (22–105)	17
−3	7	8	1.5 (0.7–3.5)	11.5	54.5 (16–129)	19
−2	11	16	1.7 (0.7–3.3)	18.5	34 (3–138)	15.5
−1	19	32	1.9 (0.0–5.5)	23	56 (5–154)	16
0	11	12	1.9 (0.7–5.1)	17.5	37.5 (4–259)	15
1	3	3	2.3 (1.2–6.3)	15	117 (54–137)	15
2	1	1	0.1	26	5	12

mixed with petechial lesions. It improved simultaneously with improvement in platelet count. These two patients were not re-treated. Of the two other patients with a rash, one was re-treated at the same dose and the other at a lower dose: there was no recurrence of the rash. One patient was mistakenly started at 400 mg/m^2. She had severe nausea, vomiting, mucositis, myelosuppression, and rash and required hospitalization for supportive care. Despite the high dose, she had progressive disease before the second cycle could be initiated and was taken off study. She also experienced photophobia and transient tinnitus, both of which were completely reversible. Eight patients received only one cycle of chemotherapy. Two of them died during this first cycle, one with nonneutropenic pneumonia, the other one with lactic acidosis. The first one was inevaluable; the second one had reached a minor response. Of the other six, all had developed clear-cut evidence of progressive disease before the second cycle could be administered. Of the 22 patients who received two or more cycles of iproplatin, five completed their treatment at their starting dose; two had one dose escalation and remained at the higher dose until being taken off study for progressive disease. Two additional patients had one dose escalation but were subsequently deescalated, while 13 patients had to be deescalated one or more levels.

Fifteen cycles of therapy were delayed because of prolonged myelosuppression: thrombocytopenia for eight patients, neutropenia for three, and both for four additional patients. Hematologic toxicity is described in table 3. Cumulative thrombocytopenia was noted. Seven patients required transfusion of packed rbcs for reversal of anemia (hemoglobin < 8 g/dl: a total of 18 units of packed rbcs were administered). Eight patients required platelet transfusions (platelet count $< 20{,}000$/dl), and 104 units of platelets were administered to this group. Petechiae and mild bruising occurred in eight patients. Two patients developed pneumonia during this study. Neither one was neutropenic at the time. One died despite appropriate antibiotic therapy, while the other recovered uneventfully. One patient who had a substantial response in a large, ulcerated, locally advanced breast cancer developed lactic acidosis during the first course; she died despite supportive care and antibiotic therapy. Two other patients died shortly after completing the study with rapidly progressive metastatic breast cancer. No changes in creatinine or BUN were seen in this study. No episodes of neurotoxicity or ototoxicity were noted.

DISCUSSION

This study again demonstrates that iproplatin can be administered on an ambulatory basis and without hydration. However, aggressive antiemetic therapy is required to make the drug tolerable. Premedication with diphenoxylate might be useful to decrease the incidence and severity of diarrhea. Iproplatin has definite antitumor activity against metastatic breast cancer, although this activity is modest. The level of activity might be higher in patients without prior chemo- or radiotherapy, as was demonstrated with cisplatin (2). The pattern of toxicity of this agent is clearly different from that of cisplatin. While it has the advantage that no nephro- or neurotoxicity is produced, gastrointestinal toxicity and cumulative thrombocytopenia are severe. Since these latter toxic effects are clearly dose dependent, it is likely that at the doses used in combination with other cytotoxic agents, gastrointestinal toxicity would be quite tolerable, especially with appropriate premedication. In this group of patients with favorable characteristics, 270 mg/m^2 was the maximum tolerable dose. We believe the activity observed justifies the evaluation of iproplatin in combination with other agents in patients with metastatic breast cancer.

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Multicenter Study of Trilostane: A New Hormonal Agent in Advanced Postmenopausal Breast Cancer[1]

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Trilostane, an inhibitor of the production of adrenal estrogens, was administered, together with dexamethasone, to 97 eligible postmenopausal women with advanced breast cancer. Seventy-four patients who had either received trilostane for a minimum of 10 weeks or whose disease had progressed while on trilostane before this period were assessed for tumor response. Eighteen patients (25%) had objective responses (two complete, 16 partial); a further 21 patients had stable disease. The response rate among all 97 patients, including those not treated for a minimum 10-week period, was 19%. Thirty-two of 97 patients reported adverse reactions which were attributed to trilostane and/or dexamethasone. Therapy was stopped for 15 patients, and the dose of trilostane was reduced for ten. Diarrhea was the commonest side effect, being reported in 16 patients, of whom nine stopped treatment. Trilostane, given with a corticosteroid, is an effective alternative hormonal agent acting by adrenal blockade for postmenopausal women with advanced breast cancer. [Cancer Treat Rep 71:1197-1201, 1987]

Trilostane (4-α-5-α-epoxy-17-β-hydroxy-3-oxoandrostane-2-α-carbinitrite) is a synthetic steroid which has been shown to specifically and competitively inhibit activity of the 3-β-hydroxysteroid dehydrogenase:δ-5,4-isomerase system, both in vitro and in vivo, in a number of species, including man (1-3). This causes inhibition of the conversion of biologically inactive steroids into their active metabolites. These include adrenal-derived androstenedione, which is the primary source of estrogens in the postmenopausal woman. It has also been suggested that trilostane may inhibit the aromatase enzyme, with resultant reduction of circulating estrone levels of postmenopausal women (4). Because it is known to inhibit production of adrenal estrogens, it has recently been tested as a hormonal agent in postmenopausal women with metastatic breast carcinoma. Beardwell et al (5) treated 23 patients with trilostane and dexamethasone and reported objective responses in six (26%) and stabilization of progressive disease in three (13%). Wilkinson et al (6), extending the experience of this group, reported responses in ten of 31 (32%) patients who had received therapy for > 8 weeks.

This multicenter study was designed to further extend experience of the degree of activity and pattern of toxicity of trilostane when used together with dexamethasone. All patients gave verbal informed consent to inclusion in the study, and ethical committee approval for the study was granted at each participating institution.

PATIENTS AND METHODS

Entry Criteria

This was an open-assessment multicenter study into which only patients fulfilling the following entry criteria were admitted. All patients were postmenopausal, defined as a minimum of 1 year since last menstrual period, at least 6 months after oophorectomy, or radiation-induced menopause. Patients > 50 years of age who had had a hysterectomy were also eligible. All patients had objective evidence of disease progression as defined by International Union Against Cancer (UICC) criteria (7). Their disease was measurable or evaluable by direct measurement, photography, radiography, or other imaging techniques.

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[2] This study was conducted by the British Trilostane Study Group. The following U.K. institutions participated in the study: Bristol Radiotherapy and Oncology Centre (*V. Barley*); Department of Medical Oncology, Queen Elizabeth Hospital, Birmingham (*G. Blackledge*); Woodend Hospital, Aberdeen (*A. Hutcheon*); Gartnavel General Hospital, Glasgow (*S. Kaye*); Victoria Infirmary, Glasgow (*D. Smith*); Velindre Hospital, Cardiff (*C. Keen*); University Department of Surgery, Welsh National School of Medicine, Cardiff (*D. J. T. Webster*); Royal Devon and Exeter Hospital (Wonford), Exeter (*C. Rowland*); Freedom Fields Hospital, Plymouth (*C. Tyrrell*); and Royal South Hants Hospital, Southampton (*C. J. Williams*).

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Hormone receptor data are not routinely available in the United Kingdom, and patient accrual was therefore not restricted to estrogen receptor (ER)-positive patients.

Patients were excluded from trial entry for the following reasons: (*a*) they had a poor performance status (eg, completely disabled or confined to bed) and an expected survival of < 3 months; (*b*) they had received any hormonal therapy in the preceding 1 month; (*c*) they had major hepatic or CNS disease; (*d*) they had inevaluable disease (one or more of the following as sole evidence of disease: lymphedema; hilar enlargement; pleural effusion; ascites; CNS metastases; marrow suppression; osteoblastic lesions); or (*e*) they had prior or current malignancies at other sites, apart from adequately treated in situ cervical carcinoma or squamous cell skin cancer.

No patient had received prior trilostane therapy.

Assessment

Patients were assessed before trial entry, at 6 and 12 weeks, and thereafter as required. Assessment included history and physical examination, photography of superficial lesions, limited skeletal survey (including chest x-ray) bone scan, routine hematology (cbc), and biochemistry (liver function, electrolytes, and renal function) together with recording of performance and pain status (both on a five-point scale). Potential drug-related effects were recorded at each clinic visit, though patients were not directly questioned about specific toxic effects.

Response Criteria

The criteria used were those proposed by the UICC (7). In brief, these included: *complete response* (CR)—disappearance of all known disease (in the case of lytic bone lesions, these had to be shown to have calcified); *partial response* (PR)—≥ 50% decrease in measurable lesions and objective improvement in evaluable but nonmeasurable lesions (it was not necessary for every lesion to have regressed provided that none of the lesions had progressed); *no change* (NC)—lesions unchanged (ie, < 50% decrease or < 25% increase in the size of measurable lesions; if nonmeasurable but evaluable lesions represented the bulk of disease and these clearly did not respond even through measurable lesions had improved, this was classified as NC); *disease progression* (PD)—described as mixed if some lesions regressed while others progressed (> 25% increase in measurable lesions). Cases in which some or all lesions progressed and/or new lesions appeared were described as failures.

Response duration was measured from Day 1 of trilostane therapy until tumor progression. Survival was also measured from first treatment.

Drug Administration

Trilostane was administered orally in the following dose schedule (each dose administered four times/day): Days 1–3, 60 mg; Days 4–7, 120 mg; Days 8–10, 180 mg; and Day 11 onwards, 240 mg. Dexamethasone was given at a dose of 0.5 mg twice a day from Day 1.

RESULTS

A total of 103 patients were entered into the study by nine institutions between October 1982 and October 1983. Six were ineligible because of protocol violations (table 1). Thirteen patients in this group who had PD before 12 weeks have been included in the denominator in calculating response rates. Response rates have been calculated both including and excluding those who did not receive trilostane for a minimum of 10 weeks, this period of time being considered the minimum needed to evaluate efficacy.

Characteristics of the patients entering the study are shown in table 2. ER data were only available for 15 patients (ER-positive, 12; ER-negative, three). Eighteen (25% of the 74 fully assessable patients or 19% of the 97 eligible patients) had an objective response (CR + PR; table 3). An additional 21 patients (28% of assessable patients or 22% of the 97 eligible patients) had stable disease (NC). The median duration of objective response was > 36.5 weeks for CRs and was 41.2 weeks for PRs; one-third (six) of these patients remain in remission.

Most patients had multiple sites of disease. The response rate for patients with evaluable soft tissue disease was 31%; for those with evaluable bone metastases, the rate was 24%. Few patients had a visceral pattern of disease; of 15 patients with either liver, lung, or pleural disease, only two (13%) responded.

Data on the patients' serial performance status were available in 77 patients; 14 (18%) showed an improvement in mobility. Twenty of 76 (29%) patients also had an improvement in their pain rating. Improvement in mobility and pain correlated well with objective response to trilostane.

When responders were compared with nonresponders,

TABLE 1.—Trial entries and withdrawals

Status	No. of patients
All patients entering study	103
Ineligible	6
Pre- and peri-menopausal	2
Concomitant tamoxifen	2
Therapy within preceding mo	1
No assessable disease	1
Withdrawal before 12 wk	36
Side effects	15
Early PD*	13
Death (non-drug-related)	4
Concomitant clinical event	2
Persistent hypercalcemia	1
Patient choice	1

*These patients were included in the denominator when calculating response rates in assessable patients. Response rates have been calculated for all 97 eligible patients.

TABLE 2.—Characteristics of 97 eligible patients

Characteristic	No. of patients
Mean age in yrs (range): 62.1 ± 10.9 SD (37-83)	
Menopausal status	
Natural menopause	61
Oophorectomy	13
Hysterectomy (> 50 yrs of age)	13
Radiation-induced menopause	9
Unknown	1
Time postmenopause (yrs)	
< 1	5
1-5	18
> 5	72
Unknown	2
Disease-free interval (yrs)	
< 1	15
< 2	46
> 2	35
Unknown	1
Previous therapy	
Surgery	71
Primary radiotherapy	28
Secondary radiotherapy	59
Chemotherapy	36
Hormone therapy*	84
None	1
Unknown	1
Site of disease	
Soft tissue	85
Lung	6
Pleura	8
Liver	3
Bone	60
Single	39
Multiple	58

*Some patients had received > 1 prior hormonal therapy.

it was found that patients obtaining an objective remission tended to be older (68.2 ± 11.2 vs 57.5 ± 9.1 years) and to have had a longer interval from menopause to the diagnosis of breast cancer (13.2 ± 11.7 vs 6.7 ± 10.2 years) and a longer interval from menopause to starting trilostane (17.9 ± 10.7 vs 11.3 ± 9.6 years). There was no correlation between disease-free interval, prior therapy, or sites of disease and response.

Of the 97 eligible patients receiving trilostane and dexamethasone, adverse reactions were reported in 44 (table 4). In 32 cases, one or more adverse reactions were attributed to either trilostane or dexamethasone. Therapy was stopped in 15 cases, and the dose of trilostane was reduced for 10 patients. The most troublesome side effect was diarrhea, reported by 16 patients, nine of whom stopped therapy; its onset was very variable, ranging from 1-287 days. Cushingoid features were common (ten patients), and one patient developed steroid-induced diabetes mellitus. Therapy was stopped in three of these patients, and the dose of dexamethasone was reduced in six. Although lying and standing blood pressure was measured at each visit, postural hypotension was found in only one patient. Nausea was reported by five patients and skin rashes by two. Two patients in the study reported facial and/or gum and tongue pain. An additional two ineligible patients who were given trilostane reported similar facial pain. There were no apparent drug-induced abnormalities in the routine biochemical and hematological parameters recorded.

DISCUSSION

Trilostane, together with a corticosteroid, inhibits the conversion of adrenal androgens to estrogen and may have an effect which is partly analogous to that of aminoglutethimide and a corticosteroid (4,8,9). This large multicenter study and other studies have shown that trilostane has a moderate antitumor effect in postmenopausal women with breast cancer who have been heavily pretreated. Twenty-five percent of assessable patients in this study achieved an objective response, a figure comparable with those previously reported from phase II studies of aminoglutethimide and a corticosteroid in similar patients (10,11).

Two earlier pilot studies of trilostane have shown conflicting results. Wilkinson et al (6) reported a 32% response rate to trilostane in 31 patients; in contrast, Coombes et al (12) reported only one PR and six cases of disease stabilization among 41 patients. However, the latter group did not give a corticosteroid with the trilostane, and this may be necessary to prevent a reflex rise in adrenocorticotrophic hormone. Furthermore, only a

TABLE 3.—Response to trilostane therapy

Response	No. of patients	% of assessable patients* (n = 74)	% of evaluable patients (n = 97)	Mean duration of response† (wks)
CR	2	3	2	> 36.5
PR	16	22	17	41.2
CR + PR	18	25	19	
NC	21	28	21	32.2
PD	35	47	60	
After > 12 wks	22	30		
After < 12 wks	13	17		
Total	74	100		

*Excludes patients who stopped trilostane within 10 wks for reasons other than PD.
†6 patients continue in objective remission.

TABLE 4.—Adverse reactions attributed to trilostane-dexamethasone

Side effect	No. of patients (n = 97)	No. of patients for whom—	
		Drug stopped	Dose reduced
Diarrhea	16	9	3
Cushingoid features	10	3	6*
Malaise/depression	4	2	—
Nausea	5	—	1
Gastritis	3	1	—
Itchy skin or rash	2	—	—
Hallucinations†	2	—	—
Facial/gum pain‡	2	—	—

*Dexamethasone dose reduced.

†Both patients were taking concomitant analgesics and antidepressants.

‡An additional 2 patients, excluded because of protocol violations, reported facial or gum pain.

minority of patients in the Coombes et al study (fewer than ten) received the full dose planned (960 mg/day). Hindley et al (13) suggested that a dose less than this (480 mg/day) is ineffective, although Coombes and his colleagues found no evidence of a dose-response effect among their patients with stable disease.

Although trilostane was stopped because of adverse reactions in 15 patients, the only commonly reported side effect was diarrhea in 16 of 97 patients. Nine of these patients discontinued trilostane. Onset of diarrhea was very variable, occurring as early as the second dose on Day 1 or as late as Day 287. It was often accompanied by colicky abdominal pain. Similar symptoms were reported in the initial human volunteer studies of trilostane and in patients treated for hyperaldosteronism/hypertension and Cushing's syndrome.[3] Beardwell et al (5) noted diarrhea in 12% of postmenopausal women receiving this drug, and severe diarrhea was reported by 5% of the patients in the study of Coombes et al (12).

Cushingoid features, seen in 10% of patients, were related to the relatively high dose of corticosteroid used. Dose reduction of dexamethasone by up to 50% did not result in disease progression in six responders continuing on trilostane. Pain in the buccal cavity and a burning sensation have been reported in human volunteer studies,[3] especially after rapid escalation of drug dose, and two of 97 patients in this study complained of similar symptoms.

Beardwell et al (5) reported asymptomatic postural hypotension, treated with fludrocortisone, in 44% of 41 assessable patients. Although lying and standing blood pressures were recorded for all patients in the current study, only one patient had postural hypotension. Wilkinson et al (6) found that ten of 41 patients (24%) experienced nausea. However, only 5% of patients in the present study complained of nausea, and none complained of vomiting.

[3]Unpublished data on file at Sterling Winthrop Research and Development.

Coombes et al (12) have suggested that the responses reported to treatment with trilostane and a corticosteroid may, at least in part, be due to the direct action of the corticosteroid. However, Hindley et al (13) reported no responses in 19 patients treated with a lower dose of trilostane (480 mg/day) despite the addition of dexamethasone (0.5 mg twice/day). The role of corticosteroids, when used in conjunction with drugs causing adrenal suppression, needs further assessment.

The potential role of trilostane is as a second-line hormonal agent after tamoxifen. Because of this, its pattern of toxicity and activity by site are important. Aminoglutethimide, which includes adrenal suppression as part of its mechanism of action, commonly produces troublesome side effects, notably sedation and skin rash. Some data, however, suggest that it is particularly effective in the treatment of osseous metastases from breast cancer (14). Although the response rates for trilostane were highest for soft tissue lesions (31%), intermediate for bone metastases (24%), and lowest for visceral disease (13%), the number of patients in each subgroup was too small to allow meaningful analysis.

Trilostane, with dexamethasone, is an active new hormonal agent in postmenopausal women with advanced breast cancer. In order to assess its place among the many hormonal drugs now available, it is being compared, in a randomized fashion, with aminoglutethimide, with special attention paid to the acceptability of treatment, side effects, and the response rate according to disease site.

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Lomustine, Etoposide, Vindesine, and Dexamethasone (CEVD) in Hodgkin's Lymphoma Refractory to Cyclophosphamide, Vincristine, Procarbazine, and Prednisone (COPP) and Doxorubicin, Bleomycin, Vinblastine, and Dacarbazine (ABVD): A Multicenter Trial of the German Hodgkin Study Group[1]

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Thirty-two patients with advanced Hodgkin's lymphoma resistant to cyclophosphamide, vincristine, procarbazine, and prednisone (COPP) and doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) were treated with a salvage chemotherapy regimen consisting of lomustine, etoposide, vindesine, and dexamethasone (CEVD). Twenty-seven patients were treated because of primary resistance to COPP/ABVD, and five patients were treated in early relapse (< 12 months) after COPP/ABVD-induced complete remission. Fourteen patients (44%) achieved complete remission, and four patients achieved partial remission, with an overall response rate of 56%. Two partial responders achieved complete remission after additional radiotherapy. Four of five patients in early relapse after COPP/ABVD achieved complete remission. Consolidation radiotherapy was given for only one complete responder. Median duration of complete remission is > 10 months, and median survival is > 26 months. The treatment was well-tolerated. The main side effects were leukopenia, thrombocytopenia, mild nausea/vomiting, and cushingoid side effects. CEVD is a very active and well-tolerated salvage chemotherapy regimen in patients with Hodgkin's disease resistant to or relapsing after COPP and ABVD. [Cancer Treat Rep 71:1203-1207, 1987]

The treatment results of advanced Hodgkin's disease have markedly improved with the advent of combination chemotherapy (1). However, 30%-50% of patients presenting with advanced stages (2,3) or in relapse after treatment with primary radiotherapy (4) require second-line chemotherapy. For Hodgkin's lymphoma resistant to mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) chemotherapy, the Milan group reported a 59% complete remission rate in 54 patients who were treated with doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) (5). When Bonadonna's group showed that the combination of MOPP and ABVD was superior to MOPP alone (6), MOPP/ABVD became the standard front-line combination chemotherapy in many centers (7,8). In a multicenter study of the German Hodgkin Study Group, complete remission rates in patients with stage IIIB/IV disease could be significantly increased after replacing COPP, a modification of the MOPP protocol in which mustargen is substituted for by cyclophosphamide (9), with a combination chemotherapy consisting of alternating cycles of COPP and ABVD (10). In stages IIIB and IV, 79 of 123 patients (62%) achieved complete remission after six cycles of COPP/ABVD. Whereas patients who are not in complete remission because of persisting nodal involvement can be salvaged by radiotherapy, those with persistent disseminated disease or organ involvement require additional chemotherapy. For this group of patients we developed a combination of lomustine (CCNU), etoposide, vindesine, and dexamethasone (CEVD). CCNU and etoposide had both shown activity in previously treated Hodgkin's lymphoma (11,12). Vindesine was chosen because patients who had received vincristine and vinblastine had responded to vindesine (13) and because vindesine is active in patients resistant to MOPP and ABVD (14). Dexamethasone was added to these three cytotoxic drugs for two reasons: first, most patients with refractory Hodgkin's disease present with B symptoms, which respond to steroids; and second, we speculated that the different pharmacokinetics and longer biological half-life of dexamethasone in comparison to prednisone might add an additional effect to the cytotoxic combination of CCNU, etoposide, and vindesine.

We now report on the results of CEVD treatment in

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TABLE 1.—Characteristics of patients treated with CEVD

Characteristic	No. of patients
Total entered	32
Total evaluable	32
Median age in yrs (range): 31 (15–53)	
Sex	
Female	17
Male	15
Stage at entry	
IIAE	1
IIIA2	1
IIIB	6
IVA	5
IVB	19
Histology	
Nodular sclerosis	16
Mixed cellularity	11
Lymphocyte predominant	3
Lymphocyte depleted	2
Prior therapy (+ radiotherapy)	
Alternating COPP/ABVD	16 (6)
Sequential COPP/ABVD	16 (10)

32 patients refractory to COPP/ABVD. Our results indicate that CEVD is an effective and well-tolerated regimen for this group of patients.

MATERIALS AND METHODS

From January 1983 to December 1986, 32 patients (17 females and 15 males) with histologically proven Hodgkin's lymphoma who had failed on COPP/ABVD chemotherapy were treated with CEVD. Patient characteristics are shown in table 1. Median age was 31 years (range, 15–53). Systemic symptoms were present in 25 patients. Eight patients presented with nodal disease, and 24 patients had extranodal disease or disseminated organ involvement. Only patients with disease resistant to COPP and ABVD were eligible. Resistance was defined as progressive or persistent disease after six cycles of chemotherapy (with or without irradiation) or relapse within 12 months of complete remission achieved with sequential or alternating COPP/ABVD. Sixteen patients had received COPP and ABVD sequentially, and 16 had received alternating COPP/ABVD combination chemotherapy. Five patients were in early relapse (3–7 months; median, 5 months) after COPP/ABVD-induced complete remission, 12 had achieved partial remission with COPP/ABVD, and 15 had experienced progressive disease during COPP/ABVD treatment.

The CEVD regimen consisted of CCNU (80 mg/m^2 orally on Day 1), etoposide (120 mg/m^2 orally or 60 mg/m^2 iv per day from Days 1 to 5 and from Days 22 to 26), vindesine (3 mg/m^2 iv on Days 1 and 22), dexamethasone (3 mg/m^2 orally on Days 1–8, 1.5 mg/m^2 orally on Days 9–26), at 42-day intervals. The choice between iv and oral application of etoposide was up to the participating institution and depended on the physician's preference. Treatment was repeated for a total of four cycles. Of the 14 complete responders, 11 received no additional therapy for consolidation, one received one and one received two additional cycles of CEVD, and only one patient received consolidation radiotherapy.

Prior to CEVD therapy, extent of disease was assessed by chest x-ray, abdominal sonogram and/or computerized tomography, bone marrow, and liver biopsy. After the end of therapy all manifestations of Hodgkin's lymphoma were reassessed by adequate methods, including pathologic restaging for patients who had had bone marrow and/or liver involvement prior to CEVD therapy.

Written informed consent was obtained from all patients. All patients who started therapy were considered evaluable for response. *Complete response* (CR) was defined as the disappearance of all measurable disease for at least 2 months after the end of treatment, and *partial response* (PR) was defined as a $> 50\%$ reduction of the measurable tumor mass and disappearance of systemic symptoms.

RESULTS

The results of therapy are summarized in tables 2 and 3. Etoposide was given iv to 19 patients and orally to 13. There was no difference in response rate or toxicity between the two groups. CR was obtained in 14 of 32 patients (44%; 95% confidence limits, 27%–61%) and PR in 4 of 32 (12%), for an overall response rate of 56% (95% confidence limits, 39%–73%). Two patients in PR after CEVD achieved CR after additional radiotherapy. Three patients showed stable disease ("no change") and 11 progressed.

Twenty-seven patients were treated with CEVD because of primary resistance to COPP/ABVD. Of those, 15 were in progression and 12 were in partial remission, minor response, or no change prior to CEVD. Five of 15 (33%) in progression after COPP/ABVD achieved CR and three (20%) achieved PR, with an overall response

TABLE 2.—Results of CEVD therapy

	No. of patients (%)
Entered	32
Evaluable	32
CR	14 (44)
Median duration in mos (range): 10+ (2–37+)	
PR	4 (12)
Median duration in mos (range): 8 (4–11)	
No change	3
Disease progression	11
Median survival in mos (range)	
Complete responders: 26+ (3–44+)	
Partial responders: 19 (13+–20)	
Nonresponders: 10 (1–31+)	

TABLE 3.—Results of CEVD treatment according to response to COPP/ABVD therapy

Response to COPP/ABVD	No. of patients
CR	
Evaluable for CEVD	5
CR on CEVD	4
PR on CEVD	1
PR, minor response, no change	
Evaluable for CEVD	12
CR on CEVD	5
PR on CEVD	0
Disease progression	
Evaluable for CEVD	15
CR on CEVD	5
PR on CEVD	3

rate of 53%. The respective figures for 12 patients in PR, minor response, or no change after COPP/ABVD were 5 of 12 CR and no PR. Thus, the response to CEVD was similar in patients in progression after COPP/ABVD and in those who had shown an incomplete response. However, the response was better in patients who had obtained CR after COPP/ABVD and were treated with CEVD in early relapse. In this group, four of five patients obtained complete remission with CEVD. There was no difference in the CR rate whether CEVD was given after sequential (16 patients) or alternating (16 patients) COPP/ABVD. In each group seven patients achieved CR and two PR, with an overall response rate of 56% in both groups.

The CR rates were achieved with four courses of CEVD. Even though there as no consolidation therapy according to the protocol, consolidation treatment was given to three patients by decision of the participating physician: one patient received consolidation radiotherapy because of prior bulky disease, and two patients received one and two additional CEVD courses. The median survival of patients with CR is > 26 months (range, 3–44+) (fig 1). The median duration of CR has not yet been reached and is > 10 months (fig 2). The median duration of PR is 8 months, while the median duration of survival of patients with PR is 19 months. The median survival of patients not responding to CEVD (no change or progression) is 10 months (range, 1–31) (fig 1).

Treatment with CEVD was well-tolerated. The main side effects are listed in table 4. They consisted of leukopenia, thrombocytopenia, alopecia, nausea/vomiting, and cushingoid side effects, including exacerbation of insulin-dependent diabetes mellitus. The mean leukocyte count nadir was $2.2 \times 10^3/mm^3$ (range, 0.2–11.3), the platelet count nadir was $130 \times 10^3/mm^3$ (range, 15–410). Despite the considerable myelosuppression, there was only one episode of septicemia, and no bleeding was observed. All side effects were reversible. Treatment had to be delayed because of leukopenia in 25% of courses and because of thrombocytopenia in 10%. Subjective tolerance of CEVD as reported by the patients was much better than that of ABVD and similar to that of COPP. Only one patient refused to complete therapy when he was in PR and free of symptoms after one cycle of CEVD because he developed a phobia about seeing infusion bottles. The percentage of the optimal dose administered was as follows: CCNU, 95% (first cycle, 96%; second, 96%; third, 92%; fourth, 97%); etoposide, 80% (102%, 84%, 72%, and 63%, respectively); vindesine, 97% (110%, 99%, 95%, and 84%, respectively); and dexamethasone, 103% (101%, 95%, 108%, and 108%, respectively).

DISCUSSION

The results of CEVD as salvage chemotherapy for patients with Hodgkin's lymphoma resistant to both

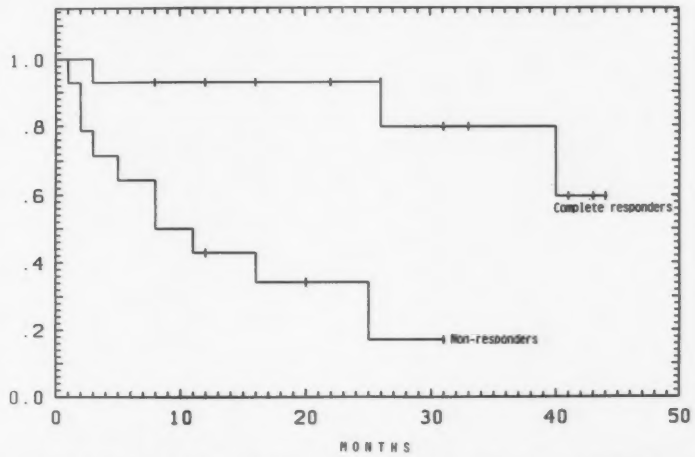

FIGURE 1.—Survival of complete responders and nonresponders (no change or disease progression) among patients treated with CEVD.

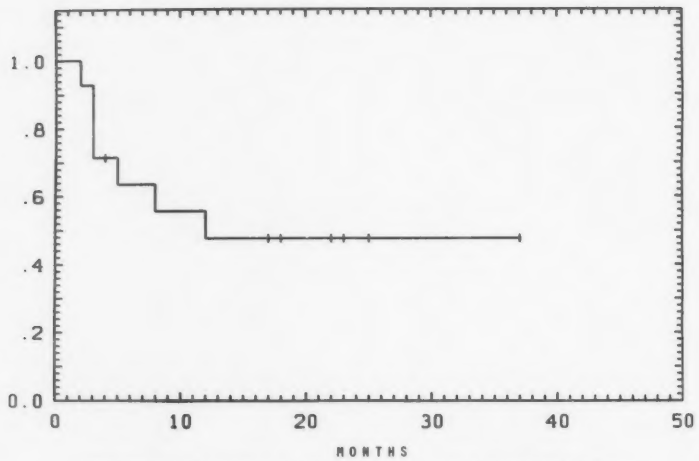

FIGURE 2.—Relapse-free survival of patients achieving CR with CEVD.

COPP and ABVD are encouraging. The fact that only one of the 14 CRs received consolidation radiotherapy demonstrates that CEVD alone is capable of inducing long-lasting remissions in heavily pretreated patients with Hodgkin's lymphoma. Since four of the complete responders relapsed with nodal disease which had not been previously irradiated, one might speculate that the duration of CR may be further improved by consolidation radiotherapy, as has been suggested by others (15).

The toxicity of the CEVD regimen was moderate and allowed for administration in an outpatient setting, as all of the drugs except vindesine can be given orally. The prompt symptomatic improvement after the initiation of CEVD may be partly due to the relatively high dose of dexamethasone delivered during the first week of the cycle. Subjective tolerance to the CEVD regimen was far better than to ABVD, mainly due to the reduced vomiting, and was reported by the patients to be similar to COPP. Grade III vomiting was observed by our group in 60% of patients after ABVD, but only in 12% after CEVD.

Whereas high-dose chemotherapy with autologous bone marrow transplantation seems to be an attractive approach for patients with resistant Hodgkin's lymphoma (16,17), the exact role of this toxic therapy in salvaging patients with refractory Hodgkin's lymphoma in comparison to conventional chemotherapy remains to be defined. The results with CEVD are in line with or better than those of recently reported third-line salvage regimens (table 5), such as CEP (CCNU, etoposide, and prednimustine) (18,19), CAD (CCNU, melphalan, and vindesine) (20), MIME (mitoguazone, ifosfamide, methotrexate, and etoposide) (21), CEM (CCNU, etoposide, and methotrexate) (22), the combination of teniposide, cisplatin, hexamethylmelamine, and prednisolone (23), or the obviously more toxic PEB/CCNU/MTX-L (cisplatin, etoposide, bleomycin, CCNU, and methotrexate with leucovorin rescue) (24). As the results of salvage chemotherapy regimens are influenced by prior therapy, disease extent, systemic symptoms, and performance status, it is difficult to compare the various salvage protocols. However, for several reasons, we believe that CEVD is one of the most active salvage protocols for Hodgkin's lymphoma resistant to both COPP (or MOPP) and ABVD: (*a*) the patients included in the CEVD study had a high level of negative prognostic factors (the low age range and median age are similar to those in the other trials listed in table 5); (*b*) in contrast to other salvage protocols, the high response rates were achieved without the addition of radiotherapy; (*c*) CRs were observed in all subgroups of patients, including those with liver or bone marrow involvement; and (*d*) the observed durations of CR were achieved without consolidation radiotherapy. The response rate of CEVD in Hodgkin's lymphoma resistant to COPP/ABVD suggests a non-cross-resistance of CEVD with COPP/ABVD which merits a trial incorporating this regimen or parts of it into front-line protocols. Such a regimen consisting of biweekly alternating application of COP/ABV/IMEP (ifosfamide, methotrexate, etoposide, and prednisone) is

TABLE 4.—Toxicity of CEVD combination chemotherapy*

Toxic effect	WHO† grade				
	0	I	II	III	IV
Leukopenia	25	16	22	25	12
Thrombocytopenia	56	6	13	13	12
Nausea and vomiting	4	60	24	12	0
Neurotoxicity	83	11	6	0	0
Alopecia	0	11	29	60	0
Cushingoid features	16	48	24	12	0

*Values = % of patients.
†World Health Organization criteria.

TABLE 5.—Results of salvage therapy in patients refractory to MOPP or COPP and ABVD*

Protocol	Ref No.	No. evaluable	CR (%)	CR + PR (%)	Mean duration (mos)		% of patients with—		
					CR	CR + PR	Leukopenia grade III + IV	Thrombocytopenia grade III + IV	Vomiting grade III + IV
CCNU, etoposide, prednimustine	18	58	40	54	15	n.m.	10	16	84
CCNU, etoposide, prednimustine	19	15	26	40	12	5.5	3	3	27
Teniposide, cisplatin, hexamethylmelamine, prednisolone	23	56	6	31	n.m.	4.8	12	14	18
CCNU, melphalan, vindesine	20	15	13	46	n.m.	n.m.	13	7	7
CCNU, etoposide, methotrexate	22	21	14	43	19	4	19	19	n.m.
Cisplatin, etoposide, bleomycin, CCNU, methotrexate	24	5	20	20	n.m.	n.m.	50	21	79
Mitoguazone, ifosfamide, methotrexate, etoposide	21	43	23	60	n.m.	n.m.	80	50	n.m.

*n.m. = not mentioned.

currently being evaluated in a pilot study by the German Hodgkin Study Group.

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Mitoxantrone in the Treatment of Patients With Non-Hodgkin's Lymphoma[1]

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Thirty-five patients with non-Hodgkin's lymphoma, who had relapsed from or failed prior cytotoxic regimens including doxorubicin, received mitoxantrone at a dose of 14 mg/m² iv every 3 weeks. According to the working formulation, 18, 15, and two patients had low-, intermediate-, and high-grade malignancy, respectively. Thirty-four patients were evaluable for response and all were evaluable for drug toxicity. Three patients achieved complete response, 12 achieved partial response, eight had stable disease, and 11 had progressive disease. The overall objective response rate was 43% (95% confidence limits, 25%–61%) for all patients. The response durations ranged from 7 to 11+ months. Time to treatment failure was 4.5 months (range, 1–10+). The responses achieved were clustered in patients with low-grade malignancy. There was a partial response in a patient who had relapsed from prior anthracyclines. A total of 155 cycles of mitoxantrone therapy were given. The median number of courses per patient was four (range, one to ten). Myelosuppression was the dose-limiting factor. Most nonhematologic toxic effects were mild. The data indicate that mitoxantrone is effective in the treatment of non-Hodgkin's lymphoma with acceptable toxicity. [Cancer Treat Rep 71:1209–1212, 1987]

Multidrug chemotherapy in contrast to single-agent therapy has proved curative in about 50% of patients with high-grade disseminated non-Hodgkin's lymphoma (NHL) (1–3). No such difference has been shown for patients with low-grade disseminated disease (4). Although these latter patients are sensitive to a variety of chemotherapeutic agents, there is no proof of a cured population. New chemotherapeutic agents with greater efficacy and less toxicity are therefore needed. Mitoxantrone, a synthetic anthracenedione derivation, has shown considerable antitumor activity in vitro and in vivo against solid tumors (5,6), leukemias (7), and NHLs (8–10). The aim of this study was to assess further the efficacy and safety of mitoxantrone in the treatment of patients with recurrent or refractory NHL.

PATIENTS AND METHODS

Patient Selection

Between October 1983 and May 1986, patients were entered in the study. Eligibility criteria included histological proof of NHL; presence of at least one measurable lesion (patients with bone lesions only were not eligible); relapse from or failure to prior chemotherapy; no radiotherapy and/or chemotherapy for at least 4 weeks before entry in the study; recovery from the toxic effects of prior radiotherapy and/or chemotherapy; performance status [World Health Organization (WHO)] of 0–2; life expectancy > 8 weeks; adequate bone marrow reserves (wbc count $\geq 3.0 \times 10^9$/L and platelet count $\geq 125 \times 10^9$/L) unless there was tumor involvement compromising the bone marrow; adequate liver function (serum bilirubin ≤ 20 μmols/L) unless the decreased liver function was due to tumor involvement; normal renal function (serum creatinine ≤ 120 μmols/L); and normal left ventricular ejection fraction (LVEF). The study was supervised by H. Noppa who controlled and collected the data and samples from the various centers.

Chemotherapy

Mitoxantrone was infused iv at an initial dose of 14 mg/m² in 100 ml of 5% dextrose solution and repeated every 3 weeks. Doses and time between dosages were modified according to the degree and duration of toxic effects observed. If the granulocyte count nadir was $\geq 1.5 \times 10^9$/L and the platelet count nadir was $\geq 100 \times 10^9$/L, then the dose was increased by 2 mg/m², regard-

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less of time to recovery. If the granulocyte count nadir was 0.5–1.4 × 10^9/L, the platelet count nadir was 50–99 × 10^9/L, and the time to recovery was < 21 days, then there was no dose change. If the granulocyte count nadir was 0.5–1.4 × 10^9/L, the platelet count nadir was 50–99 × 10^9/L, and the time to recovery was ≥ 21 days, then the dose was decreased by 2 mg/m² after toxicity cleared. If the platelet count nadir was < 50 × 10^9/L, then the dose was decreased by 4 mg/m² after toxicity cleared, regardless of the time to recovery. At least two cycles of treatment were to be given. Treatment was given according to the degree of response and clinical judgment. Treatment was discontinued if there was disease progression or unacceptable toxicity or the patient refused to continue the treatment. Concomitant radiotherapy was not allowed.

Assessment of Response and Toxicity

The histological classification and categorization as low-, intermediate-, and high-grade malignancy were performed using the definitions of the working formulation (11). Tumor measurements were obtained by physical examination and chest radiography every cycle and by bone marrow aspiration, ultrasonography, and isotope or computed tomography scans every three to four cycles. Initial response was assessed after the completion of the first two cycles of treatment and then subsequently. The hematologic parameters were done once a week. Electrolytes and kidney and liver function tests were done every 3 weeks. Electrocardiogram and cardiac scintigraphy and/or sonography techniques for determination of LVEF were done prior to therapy and repeated at intervals of three to four cycles. Side effects were graded according to WHO criteria (12). Cardiotoxicity was assessed according to criteria described by Alexander et al (13).

Statistical Methods

Standard statistical methods were used to calculate medians, frequencies, and confidence limits. Durations of response and time to treatment failure were calculated according to the method of Kaplan and Meier (14).

RESULTS

Patients

Thirty-five patients were entered in the study; four patients were ineligible, three due to having an abnormal baseline LVEF and one due to a performance status > 2. One patient was nonevaluable for efficacy due to protocol violation (treatment with other cytostatic agents during the second cycle of therapy). Thus, 34 patients were evaluable and 31 of these patients were eligible and evaluable. Patient characteristics are shown in table 1. Distribution of histological subgroups according to the working formulation is shown in table 2.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Entered	35
Median age in yrs (range): 65 (36–83)	
Sex	
Males	22
Females	13
Performance status	
0	15
1	17
2	2
3	1
Median No. of disease sites (range): 5 (1–11)	
No. of prior drug regimens	
< 2	24
≥ 3	11
Prior objective response to	
Radiotherapy (n = 14)	11
Chemotherapy (n = 35)	26
Anthracyclines (n = 14)	9
Median cumulative dose in mg/m² (range): 55 (13–154)	

Dosage

A total of 155 cycles of mitoxantrone therapy were given to 35 patients. The median number of treatment cycles was four (range, one to ten). The median cumulative dose was 55 mg/m² (range, 13–154). An initial dose of 14 mg/m² was given to 29 patients and 12 mg/m² was given to six patients.

Response

Of the 35 entered patients, three (9%) achieved complete response and 12 (34%) achieved partial response. Thus, the overall objective response rate was 43% (95% confidence limits, 25%–61%) for all patients. The responses achieved were in the low- and intermediate-grade malignancies. The response durations ranged from 7 to 11+ months. The median time to treatment failure was 4.5 months (range, 1–10+). Partial response was achieved in a patient who had relapsed from prior anthracyclines. There were five partial responses in patients who had failed prior chemotherapy. Three complete and seven partial responses were seen in patients who had relapsed from prior chemotherapy.

Toxicity

Nonhematologic

The nonhematologic toxic effects observed, recorded by the maximum grade per patient, are shown in table 3. Nonhematologic toxicity did not account for any decreases in dose. The majority of these toxic effects were mild. Nausea and transient vomiting were noted in 13% and 9%, respectively, of all cycles given. Only mild hair loss was observed, which occurred in 9% of

TABLE 2.—Response to mitoxantrone therapy

Working formulation	No. of patients	Response to treatment			
		Complete	Partial	Stable disease	Disease progression
Low-grade malignancy					
Diffuse, small lymphocytic	11	0	8	1	2
Follicular, small cleaved cell	4	1	2	0	1
Follicular, mixed cell	3*	0	0	2	0
Intermediate-grade malignancy					
Follicular, large cell	3	0	1	1	1
Diffuse, small cleaved cell	7	1	1	2	3
Diffuse, large cell	5	1	0	2	2
High-grade malignancy					
Large cell, immunoblastic	2	0	0	0	2
Total	35	3	12	8	11

*1 patient not evaluable for response.

patients. Moderate stomatitis was noted in 6% of patients. There were no renal or hepatic toxic effects noted. One patient died from fulminant hepatitis due to cytomegalovirus infection. Another patient died from massive gastrointestinal hemorrhage from an extensively disease-involved site during a period of drug-induced thrombocytopenia.

Hematologic

Peripheral blood cell counts were evaluable at weekly intervals during the treatment period. The frequency distributions of the lowest intracycle values of hemoglobin, granulocyte, and platelet counts are shown in table 4. The hemoglobin, granulocyte, and platelet counts were suppressed to WHO grade ≥ 3 in 6%, 48%, and 14%, respectively, of all cycles of treatment.

Cardiotoxicity

Two patients developed overt left ventricular failure during the treatment period. One patient died in congestive heart failure after a cumulative mitoxantrone dose of 27 mg/m^2. Autopsy showed this to be due to radiation-induced hemorrhagic exudative pericarditis with cardiac tamponade. There was no evidence of mitoxantrone-related cardiotoxicity. Another patient developed mitoxantrone-related congestive heart failure after a cumulative mitoxantrone dose of 154 mg/m^2. There was electrocardiographic and clinical evidence of prior atherosclerotic heart disease. The patient had also received prior chemotherapy containing doxorubicin (cumulative dose, 193 mg/m^2). The patient responded well to treatment with digitalis and diuretics. Three additional patients developed a decrease in LVEF of $\geq 0.10\%$ to a final level $> 0.50\%$ after cumulative doses of 60, 42, and 24 mg/m^2 of mitoxantrone. One patient had received prior chemotherapy containing doxorubicin (cumulative dose, 380 mg/m^2). The decreases in LVEF were considered to be related to mitoxantrone therapy.

TABLE 3.—Nonhematologic toxicity (maximum grade/patient) of mitoxantrone*

Toxic effect	WHO grade—				
	0	1	2	3	4
Nausea/vomiting	22	6	7	0	0
Constipation	34	1	0	0	0
Diarrhea	32	2	1	0	0
Abdominal pain	30	1	2	2	0
Stomatitis	33	0	2	0	0
Alopecia	32	3	0	0	0
Cardiac	33	0	0	1	1
Miscellaneous	31	3	0	0	1

*Values = No. of patients.

Off Study Reasons

Eleven patients left the study due to disease progression, four patients due to death, four due to unacceptable cardiotoxicity, and one due to prolonged thrombocytopenia. One patient refused to continue the treatment for personal reasons. There was no drug-related toxicity in this patient. Fourteen additional patients were taken off study due to lack of indication for further therapy.

DISCUSSION

The patients selected for this study had disseminated NHL with disease progression after first-line cytotoxic regimens. In such patients the response rate for mitoxantrone in comparison to other chemotherapeutic agents, eg, doxorubicin, cyclophosphamide, and chlorambucil, is of comparable magnitude (4,15–17). The present results conform to results found by others in similar patients (8,9). Currently, patients with low-grade NHL are usually treated conservatively (4), ie, treatment is withheld or discontinued if patients are asymptomatic and without major threats from the site or bulk of their lymphadenopathy. In the present study patients in this category were treated according to these principles.

TABLE 4.—Hematologic toxicity of mitoxantrone

Hemoglobin nadir (g/L)	Toxicity (% of cycles)	Granulocyte count nadir ($\times 10^9$/L)	Toxicity (% of cycles)	Platelet count nadir ($\times 10^9$/L)	Toxicity (% of cycles)
> 110	56	≥ 2	22	≥ 100	61
96–109	26	1.5–1.9	7	75–99	12
80–94	12	1.0–1.4	23	50–74	13
65–79	5	0.5–0.9	34	25–49	11
< 65	1	< 0.5	14	< 25	3

Clinically, satisfactory single-agent activity was clustered, as expected, mainly in low-grade malignancies. It is therefore apparent that mitoxantrone is useful as single-agent therapy in this group of patients with NHL.

Conventional treatment of patients with intermediate- and high-grade disease is with combination chemotherapeutic regimens (1,2). Since mitoxantrone evidently has activity in these groups of patients (9), it should be considered for incorporation into multidrug schedules and evaluated in a prospective randomized fashion in first-line patients with intermediate- and high-grade malignancies. Similarly, its contribution in salvage regimens should be evaluated. Such studies are presently in progress. These studies would allow assessment of the relative efficacy and toxicity of mitoxantrone. Due to the favorable toxicity profile of mitoxantrone (6,18), its incorporation into such treatment regimens may reduce long- and short-term side effects.

The dose-limiting toxicity of mitoxantrone is myelosuppression (6,18), eg, neutropenia which is predictable and transient. The nonhematologic toxic effects were generally mild, consisting essentially of nausea/vomiting and mild hair loss. The toxicity profile thus resembles that expected from trials of mitoxantrone in advanced breast cancer (6,18). The incidence of myocardial toxicity also appears to be of the same order as noted in breast cancer studies (18).

The data indicate that mitoxantrone has effect on NHL with acceptable toxicity in these heavily pretreated patients.

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Controlled Clinical Trial of Doxorubicin and Tamoxifen Versus Doxorubicin Alone in Hepatocellular Carcinoma[1,2]

Walter M. Mella, Philip J. Johnson, and Roger Williams[3,*]

Following reports that specific estrogen receptors could be detected in samples of hepatocellular carcinoma tissue, a prospective randomized controlled trial was undertaken in 59 patients, half of whom received doxorubicin (60 mg/m^2 at 3-week intervals) and half doxorubicin and tamoxifen (10 mg twice per day). Response occurred in three (11%) of those patients receiving doxorubicin alone and in four (16%) of those given both drugs. This difference was not statistically significant nor was the difference in survival when compared by life-table analysis. One patient treated with both drugs achieved complete remission for 30 months which was maintained (on tamoxifen alone) for 18 months before death from a non-tumor-related condition. Tamoxifen may have a role in maintenance of doxorubicin-induced remissions. [Cancer Treat Rep 71:1213-1216, 1987]

In our initial series doxorubicin was found to induce remission in 32% of patients with hepatocellular carcinoma (HCC) who were unsuitable for surgical resection (1), and experience in > 100 further patients seen in the Liver Unit and at other centers has confirmed these results (2-4). Response rates appear significantly better in those patients without underlying cirrhosis (5). There is the problem of those patients who relapse after an initial response as well as the significant percentage of patients who do not respond to doxorubicin and those who have reached the maximum dose of doxorubicin allowable without risk of cardiotixicity. The detection of specific estrogen receptors in samples of HCC tissue (6-9) has suggested, by analogy with the situation in breast cancer, an approach to treatment based on antihormone drugs. Other evidence supporting the role of estrogen in the pathogenesis and progression of this tumor is the increasing number of reports of HCC and adenomas in women taking oral contraceptive preparations (10,11).

In this study, we have compared the efficacy of doxorubicin alone with doxorubicin and tamoxifen (an antiestrogen) together in a controlled clinical trial involving 59 patients with HCC.

PATIENTS AND METHODS

The 59 patients comprised 51 males and eight females. Ages ranged from 20 to 73 years, with 36 (61%) patients > 50 years of age. The diagnosis of HCC was based on histological examination of tumor tissue in 49 patients and in nine of the remaining ten a markedly raised serum alpha-fetoprotein (AFP; 257-187,000 ng/ml; normal range, 0-10) and characteristic tumor circulation on arteriography or echodense lesion on ultrasound examination. The remaining patient had arteriographic evidence of tumor formation in the presence of HBsAg-seropositive cirrhosis. None of the patients underwent laparotomy while in the Liver Unit and tissue was therefore available for estrogen receptor analysis in any instance.

In 39 of 59 patients an underlying cirrhosis was confirmed histologically, 11 had no underlying liver disease, and in the remaining nine the diagnosis of cirrhosis could be neither proved nor excluded (table 1). The etiology of the cirrhosis was alcoholic (six patients), cryptogenic (15 patients), chronic active hepatitis (17 patients), and primary biliary cirrhosis (one patient). Twenty-three patients were HBsAg seropositive and anti-HBs or anti-HBc or both were detected in the serum of an additional 17 patients. In ten of the cirrhotic patients the tumor had not been suspected clinically and the diagnosis was made during the course of hospital admission for treatment of hepatic decompensation. Seven of these patients had raised serum AFP levels, one a filling defect on liver scan, one a blood-stained ascitic tap, and in one HCC was an unsuspected find on liver biopsy.

Patients were randomly allocated (using a system of sealed envelopes) to receive either iv doxorubicin alone [60 mg/m^2, reduced as previously described if hyper-

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TABLE 1.—Patient characteristics*

Characteristic	No. of patients receiving— Doxorubicin	Doxorubicin + tamoxifen
Entered	30	29
Evaluable	28	25
Median age in yrs (range)	53 (20-73)	51 (20-70)
Sex		
Male	25	25
Female	5	4
Underlying cirrhosis	19	18
Previous embolization	5	1
Serum HBsAg positive	12	11
Median symptom duration in mos (range)	2 (0-36)	2 (0-24)
Median survival in mos (range)	2.0 (5-26)	2 (0.5-26)
Response rate (%)	11	16

*Unless otherwise specified, values = No. of patients. 18 of the patients who received doxorubicin and 17 of those who received doxorubicin + tamoxifen have died.

bilirubinemia or leukopenia was present (1)] or doxorubicin and oral tamoxifen (10 mg twice per day).

In six patients selective percutaneous arterial embolization was carried out to decrease tumor bulk prior to starting chemotherapy. All patients gave informed consent before being entered in the trial.

Assessment of Response

Assessment of response was carried out at 3-week intervals from the start of treatment and a response was defined as one of the following: at least a 30% reduction in hepatomegaly as assessed clinically by distance below costal margin; and at least a 50% reduction in tumor diameter on serial ultrasound examinations in patients with a solitary mass or at least a 50% fall in the serum AFP level (where this was > 250 ng/ml) found at the time of presentation provided that there was no contradictory evidence from the other two criteria. If the AFP fell by $> 95\%$ and the patient lost all signs and symptoms related to the tumor then the remission was considered complete.

RESULTS

On entry in the trial, patients from the two treatment groups were comparable with respect to age, sex, symptom duration, presence of underlying cirrhosis, and HBsAg seropositivity (table 1). In five patients in each group the diagnosis had been made incidentally, and although five treated with doxorubicin alone had first been treated by embolization compared to only one in the combined-treatment group, this difference was not significant ($\chi^2 = 2.53$, $P > 0.1$). Dose adjustment was made with equal frequency in both groups, although the overall cumulative dose of doxorubicin was slightly greater in those treated with doxorubicin alone (3464 vs 2805 mg/m^2). A total of six patients were not evaluable. One patient underwent orthotopic hepatic transplantation within 1 week of entering the trial after initially declining surgery. Another patient withdrew from the trial to seek alternative therapy. The remaining four patients who were from overseas were lost to follow-up.

The median survival for those treated with doxorubicin was 2 months and for those receiving doxorubicin and tamoxifen was 2.5 months; the 95% confidence interval for the ratio of median survival (0.8) is 0.41-1.55 (fig 1). Three (11%) of the 28 patients available for follow-up examination in the doxorubicin-treated group showed a response. All three patients had raised serum AFP levels which fell to a minimum of 11%, 21%, and 36% of their initial values during treatment. Two of these patients relapsed at 3 and 8 months, while the other is still in remission at 5 months. In comparison, four (16%) of the 25 patients treated with doxorubicin and tamoxifen in whom follow-up was obtained showed a response: all had elevated serum AFP levels at presentation which fell to, within the normal range, 25%, 32%, and 16% of their presentation values during treatment. One of these patients subsequently relapsed at 8 months and two remain in remission at 5 and 2 months. All seven patients responding had a decrease in liver size as assessed clinically, and in six of these the decrease in subcostal span was $> 30\%$. The fourth patient, a 68-year-old man in whom HCC had developed as a complication of alcoholic cirrhosis, died from a myocardial infarction 30 months after presentation while in complete remission. In addition to loss of pain and other symptoms he regained his normal weight and liver size returned to normal. Serum AFP which had fallen to normal after 11 weeks of treatment remained normal for the last 27 months of his life. Doxorubicin was discontinued after 14.5 months and remission maintained with tamoxifen alone (fig 2).

Of the seven patients (six males, one female) from both groups in whom a remission was achieved, four were cirrhotic and three (one female) were noncirrhotic. The single female patient who achieved remission with doxorubicin alone had a history of oral contraceptive usage. Thus, the overall remission rate for the cirrhotic group was 10% and for the noncirrhotic group was 23%.

Apart from the patient described above who underwent complete remission, the remaining nine patients in whom the diagnosis of HCC was made incidentally were all dead within 3 months.

DISCUSSION

Since this trial was started, Friedman et al (7) have reported remission in two of five patients given progestins (megestrol or methoxyprogesterone acetate). The same group has also reported that the combination of doxorubicin and tamoxifen extended the median survival of 12 patients to > 8 months and suggested that this combi-

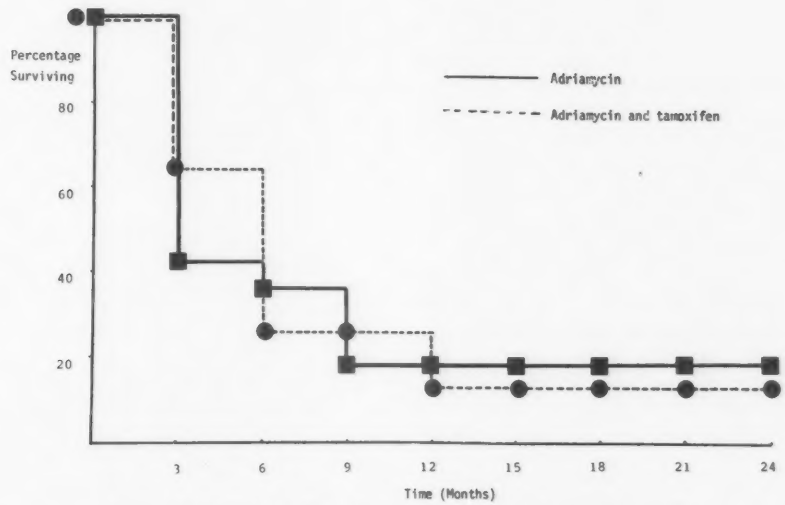

FIGURE 1.—Life-table analysis comparing survival in the 2 treatment groups.

nation might be superior to either agent used singly (12). In the present study, although there was a slightly higher response rate in those receiving the combination of drugs, the median survival time was identical in both groups as was life-table analysis of survival. In both groups, the response rate to doxorubicin was poorer than in the two previous studies from this unit when rates as high as 32% have been obtained (1,13). This may be because of the high proportion of patients with an underlying cirrhosis. These we have previously found to have a particularly poor prognosis. In a retrospective series of 150 patients with HCC, 100 of whom had underlying cirrhosis, the response to chemotherapy in the two groups was 30% and 10%, respectively (5). The corresponding figures in this trial of 23% and 10% are not significantly different. The numbers of patients without underlying cirrhosis in this study were too small to draw any firm conclusion about the efficacy of tamoxifen in this group.

The single patient who achieved complete remission after receiving doxorubicin and tamoxifen is of particular interest. In > 100 previous patients treated with

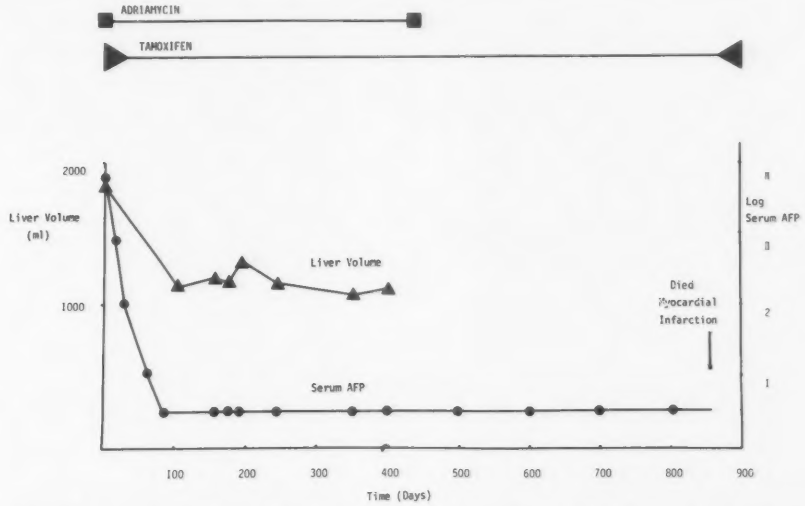

FIGURE 2.—Serum AFP and liver volume measured serially in a patient who had complete clinical remission. Liver volume was measured by ultrasound (16) and normal liver volume expected on the basis of body weight was 1350 ml.

doxorubicin alone, we have never before seen a patient in whom AFP levels have fallen to within the normal range and without exception all the complete responders have subsequently relapsed following drug withdrawal.

The observation that this patient was maintained in clinical remission for 14 months on tamoxifen alone raises the possibility that tamoxifen may have a role in maintaining rather than inducing remission, although the number of patients needed to test such a hypothesis would be very large. This would be consistent with recent studies in patients with breast cancer which suggest that tamoxifen may increase the disease-free interval following mastectomy (14).

Although a number of groups have now confirmed the presence of estrogen receptors in hepatic tumor (6–9) studies in our unit, the results of Friedman et al (7) show that similar amounts of estrogen receptors are present in normal liver tissue (8,9). This is in marked contrast to the situation in breast cancer where malignant tissue has significantly higher concentration of estrogen receptors than normal breast tissue. Furthermore, the presence of estrogen receptors does not necessarily imply that a tissue is estrogen-responsive. Studies in the rat, while confirming that the liver may be estrogen-responsive, have not confirmed that these responses are receptor-mediated (15). In our most recent studies we have shown the presence of high-affinity androgen receptors in both nuclear and cytoplasmic fractions of HCC tumor tissue, which were not detectable in normal liver (6). This suggests that the androgen receptor might be a more appropriate target for antihormonal chemotherapy.

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Phase I Study of Echinomycin[1,2]

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Echinomycin was administered to patients with advanced carcinoma in escalating doses ranging from 60 to 1500 $\mu g/m^2$ given weekly by 15-minute iv infusions for four doses, with a subsequent 2-week rest period. Dose-limiting nausea, vomiting, and anorexia associated with varying degrees of renal and hepatic dysfunction proved dose-limiting at the 1500-$\mu g/m^2$ level. Thrombocytopenia was noted in 15% of patients receiving $\geq$ 700 $\mu g/m^2$ and was severe in 11% without an evident dose-response relationship. Leukopenia was rare and mild when encountered. Allergic reactions were observed. Phase II trials are feasible using a dose schedule of 1200 $\mu g/m^2$/week $\times$ 4 weeks. [Cancer Treat Rep 71:1217-1219, 1987]

Echinomycin is a fermentation product derived from *Streptomyces echinatus*. Echinomycin acts as a bifunctional DNA intercalator resulting in inhibition of DNA-directed RNA synthesis (1,2). DNA strand breaks were not noted (2). Preclinical in vivo activity was observed against the murine P388 leukemia and B16 melanoma model systems (3,4). Preclinical toxicology studies in dogs demonstrated prominent, dose-related gastrointestinal toxic effects (emesis, diarrhea) associated with laboratory and histologic evidence of hepatic and renal dysfunction (5). Myelosuppression was not prominent or consistent in mice or dogs and no cardiac toxic effects were observed (5). Kuhn et al (6) reported in vitro activity of echinomycin against human breast and colorectal carcinomas and against human sarcomas with evidence of cross-resistance with doxorubicin in 78% of specimens previously unexposed to anthracyclines.

In view of the preclinical toxic effects, a phase I study employing a weekly $\times$ four schedule was designed. This schedule was chosen in an attempt to deliver a higher total dose with controllable gastrointestinal toxic effects. The starting dose of 60 $\mu g/m^2$ represents 1/10 of the single dose LD10 of echinomycin in mice (5).

MATERIALS AND METHODS

Patient eligibility requirements include the following: age $\geq$ 14 years; histologically proven solid tumors with convincing clinical or radiographic evidence of uncontrollable locoregional tumor or dissemination; no known treatment of higher potential efficacy; projected survival time $\geq$ 6 weeks; off of all anticancer treatments for a sufficient time to have recovered from any toxic effects; signed informed consent in compliance with institutional and Department of Health and Human Services guidelines; total wbc and platelet counts $\geq$ 4000 and 100,000/mm^3, respectively; serum bilirubin and creatinine $\leq$ 2.0 mg/dl; and no prior myocardial infarction or congestive heart failure. Measurable lesions were not required. Pregnant women were excluded. Echinomycin was supplied by the National Cancer Institute in 400-μg vacuum dried vials which were stored at $-10°C$. Solutions were prepared by adding 0.2 ml of cremophor EL and absolute ethanol (50/50 volume basis) to the vials at room temperature and agitated. After dissolution was complete, 1.8 ml of sodium chloride for injection, USP, was added, yielding a solution containing 200 μg/ml of echinomycin with 5% cremophor EL and 5% ethanol. The solutions were administered within 8 hours of preparation by a 15-minute iv infusion.

Required tests prior to treatment included: cbc with wbc differential and platelet count; a multichannel chemistry profile (SMA-12/17) which included serum electrolytes, BUN, calcium, uric acid, total protein and albumin, SGOT, LDH, alkaline phosphatase, creatinine, and bilirubin; chest x-ray and other radiologic procedures appropriate to define extent of malignancy; and an ECG. Physical examination with assessment of subjective problems and toxic effects and recording of lesion measurements, performance status, and weight were required prior to and monthly during treatment with appropriate radiologic monitoring of tumor status. Cbc, platelet count, and SMA-12/17 were to be done weekly.

Southwest Oncology Group (SWOG) toxicity criteria were employed. Liver enzyme elevations were evaluated as grade 0 toxicity (normal to 2 $\times$ normal); grade 1 tox-

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icity (2–4.9 × normal); grade 2 toxicity (5–10 × normal); and grade 3 toxicity (> 10 × normal.) Nausea and vomiting were graded as mild (less than two emesis episodes/day); moderate (between two and six episodes/day); severe (greater than six episodes/day); and life-threatening (greater than six episodes/day causing profound dehydration). Thrombocytopenia was graded as mild (75,000–99,999 cells/mm^3); moderate (50,000–74,999 cells/mm^3); severe (25,000–49,999 cells/mm^3); and life-threatening (< 24,999 cells/mm^3). Similarly, leukopenia was graded as life-threatening (wbc count < 1000/mm^3); severe (1000–1999/mm^3); moderate (2000–2999/mm^3); and mild (3000–3999/mm^3).

Groups of three or more evaluable patients were entered at each of the following levels ($\mu g/m^2$/week × 4 weeks): 60, 120, 180, 350, 525, 700, 1000, 1200, and 1500. Three patients received dose escalations after relatively low dose courses (120 and 350 $\mu g/m^2$); the remainder of patients who continued receiving treatment after the first course (4 weeks on echinomycin followed by a 2-week rest period) received their initial dose provided that tumor progression was not observed and that toxic effects resulting from the previous course had resolved. Moderate or severe toxic effects (other than nausea and vomiting) resulted in one or two dose level reductions while life-threatening toxic effects resulted in discontinuation of echinomycin.

RESULTS

Forty-nine patients were treated with echinomycin. No complete or partial responses were observed among the 42 evaluable patients with measurable lesions. Three patients with metastatic sarcomas had disease stabilization for > 3 months. Patient demographics and types of cancers treated are listed in table 1. Mild to moderate nausea was noted at all dose levels to 700 $\mu g/m^2$. At levels of 1000 and 1200 $\mu g/m^2$, moderate to severe nausea and vomiting was common and led to refusal of further treatment in two of four patients. At 1500 $\mu g/m^2$, this toxic effect became characterized as both severe and greatly protracted and often resulted in dehydration requiring parenteral support on an inpatient basis. Prerenal azotemia was noted on readmission in two of five of the patients at this level and in one patient was severe and proved fatal despite vigorous medical support. Both cases of azotemia were judged to be prerenal in origin. All five of the patients receiving 1500 $\mu g/m^2$ also had chemical evidence of hepatic damage (severe in the patient with severe nausea and prerenal azotemia), which was reversible in all patients. This toxic effect was less frequent and severe at lower dose levels as shown in table 2.

Autopsy was performed on a patient with renal and hepatic dysfunction after receiving 1500 $\mu g/m^2$. Kidneys were normal. Extensive necrosis was seen in the liver in both the hepatic parenchyma and the extensive hepatic metastases. There was no evidence of biliary obstruction.

Nausea and vomiting was most pronounced in the patients with enzyme elevations. This, coupled with the often delayed nature of nausea and vomiting, suggests that the hepatic damage may have contributed to the protracted nausea and anorexia. We consider the toxic effects at the 1500-$\mu g/m^2$ level to be dose-limiting.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Prior chemotherapy: 94%	
Median Karnofsky performance status (range): 75 (30–100)	
Median age in yrs (range): 57 (17–73)	
Sex	
Male	30
Female	19
Type of cancer	
Colorectal adenocarcinoma	12
Ovarian adenocarcinoma	2
Breast adenocarcinoma	1
Kidney adenocarcinoma	1
Stomach adenocarcinoma	1
Uterine adenocarcinoma	1
Unknown adenocarcinoma	2
Lung	
Squamous cell carcinoma	5
Large cell anaplastic carcinoma	1
Small cell anaplastic carcinoma	1
Anal, squamous cell carcinoma	1
Skin, melanoma	1
Sarcomas	12

Myelosuppression was not consistently noted at any dose level, although sufficient numbers of patients with leukopenia or with thrombocytopenia were seen at the 525-, 700-, 1000-, and 1200-$\mu g/m^2$ levels to require entry of additional patients at these levels to better define safety at these levels prior to dose escalations. No patient's leukocyte count nadir was < 3000/μl and no dose relationship is evident. Life-threatening thrombocytopenia was observed in one of seven and one of eight patients receiving 700 and 1200 $\mu g/m^2$, respectively. This was probably associated with disseminated intravascular coagulation (DIC) at 1200 $\mu g/m^2$. Risk factors predictive for severe thrombocytopenia were not evident. The inconsistency of this effect precludes us from judging thrombocytopenia to be a dose-limiting toxic effect.

Allergic reactions were noted in a patient after 25 doses of echinomycin at 525 mg/m^2 (rash) and in a second patient after the second dose of echinomycin at 1200 mg/m^2 (hypotension and wheezing), requiring discontinuation of echinomycin. Two additional patients noted transient symptoms (pruritus, flushing, and vomiting) during infusions of echinomycin, which did not recur with subsequent doses. It is not known if these reactions were caused by echinomycin and/or by the solvent system.

DISCUSSION

Echinomycin at a dose of 1500 $\mu g/m^2$/week × 4 produced dose-limiting findings and symptoms including

TABLE 2.—Toxic effects associated with echinomycin

Dose in mg/m²/wk × 4 (No. of evaluable patients)	Nausea and vomiting				Liver enzyme elevations				No. of patients with renal dysfunction	No. of patients with—					
	Mild or none	Moderate	Severe	Life-threatening	0	1	2	3		Allergic reactions	Thrombocytopenia				
											None	Mild	Moderate	Severe	Life-threatening
60 (3)	2	1	—	—	3	—	—	—	0		2	—	1	—	—
120 (5)	3	2	—	—	5	—	—	—	0		5	—	—	—	—
180 (3)	2	—	1	—	3	—	—	—	0		3	—	—	—	—
350 (4)	2	1	1	—	4	—	—	—	0	1*	4	—	—	—	—
525 (7)†	4	3	—	—	6	1	—	—	0	1‡	7	—	—	—	—
700 (7)	4	3	—	—	5	2	—	—	1		4	2	—	—	1
1000 (6)§	1	3	2	—	3	1	1	—	0		3	—	1	1	—
1200 (8)	2	3	3¶	—	6	0	1	1	2	1**	7	—	—	—	1
1500 (5)	—	1	3††	1‡‡	1	2	1	1	2††		5	—	—	—	—

*Mild pruritus during infusion, reversed without treatment.
†Includes 2 patients escalated from 350-μg/m² level.
‡Facial flushing, vomiting, felt "warm all over," reversed with treatment.
§1 partially evaluable, early death.
¶In 1 patient, none during 1st course, severe during 2nd course.
**Hypotension and wheezing, dyspenic, reversed with treatment.
††Prevented in short term by metoclopramide, but characterized as lasting days to weeks and requiring parenteral support.
‡‡Dehydration due to severe vomiting associated with hepatic enzyme elevation led to renal dysfunction, ultimately fatal.

delayed, often protracted nausea and vomiting, protracted anorexia, and reversible elevations of hepatic enzymes. Two patients at this level required cessation of treatment after two doses (one of these ultimately died of associated azotemia). A third patient's treatment was discontinued after three doses, and only two patients were able to complete the planned course of four doses. These toxic effects were less striking and generally tolerable at the 1000- and 1200-μg/m² dose levels; the latter is recommended as a starting dose schedule for patients with good performance and nutritional status and normal liver function tests. We are unable to define subsets of patients at risk of hepatic injury and protracted vomiting. The presence or absence of liver metastases and/or slightly elevated enzymes was not predictive. Only one of 12 patients with enzyme elevations had received previous nitrosoureas and none had received irradiation to any part of the liver. Phenothiazines were not used to excess and, in fact, proved relatively ineffective as antiemetics in these patients. The increasing frequency and severity of enzyme elevations with increasing doses of echinomycin, as shown in table 2, strongly suggests a dose-hepatic toxicity relationship for this drug. We cannot define a reasonable dose for patients with impaired hepatic function and in view of the toxic effects described, such patients should probably not be exposed to echinomycin, at least with this weekly × 4 schedule. Hepatotoxicity has not been reported with intermittent single doses up to 2128 and 1200 μg/m² (6,7).

Thrombocytopenia was noted in four patients (possibly and probably associated with DIC in the two life-threatening episodes) and mild leukopenia developed in an additional four patients. No dose-response relationships or factors (including types of prior therapy) predictive of myelotoxicity were noted and these toxic effects are judged to be infrequently dose-limiting. Allergic reactions as described above must be watched for with appropriate treatments readily available.

Three patients with metastatic sarcomas had disease stabilization and received 12, 15, and 25 doses of echinomycin. The latter patient's treatment was terminated due to an allergic reaction (rash). All patients had received and were refractory to previous doxorubicin. Kuhn et al (6) have noted a partial remission in a patient with metastatic fibrosarcoma. Further evaluation of echinomycin in sarcomas should be pursued.

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Doxorubicin Distribution in Human Breast Cancer[1,2]

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Using a high-performance liquid chromatographic method coupled with fluorimetric detection, we evaluated plasma pharmacokinetics of doxorubicin (DX) and tissue distribution in seven patients suffering from locally advanced breast cancer. Tumor biopsies were performed 30 minutes and 24 hours after DX injection. In addition at 48 hours, during surgery, biopsies were obtained from primary breast cancer, nodes, and other accessible tissues, and DX concentrations were analyzed. A triexponential equation gave the best fit for plasma levels and the values (mean ± SE) were: elimination half-life, 37.6 ± 4.9 hours; volume of distribution, 605 ± 61 L/m²; and clearance 200 ± 27 ml/min/m². There was greater interindividual variability in tumor DX concentrations than in plasma concentrations. DX reached much higher (range at 48 hrs, 1.54–14.17 μg/g) and longer-lasting concentrations in tumor than in plasma. At 48 hours tumor concentrations were 55.2–337.4 times the plasma concentrations. DX concentrations in normal breast were lower or similar to those in breast carcinoma. DX levels were very low in fat and skin, slightly higher in muscle, and very high in normal or metastasized lymph nodes. [Cancer Treat Rep 71:1221–1226, 1987]

Many studies have been published on the plasma pharmacokinetics of doxorubicin (DX) in man. After iv administration plasma DX levels drop quickly because the drug distributes rapidly and extensively in tissues (1–5). In mice, DX concentrations are generally high in tissues, the highest being reported in kidney, liver, and spleen (6). From some neoplastic tissues (eg, Lewis lung carcinoma) the drug is cleared very slowly compared to most normal tissues (6).

Not much information is available on the distribution of anticancer agents, particularly on DX distribution in human neoplastic and normal tissues. More knowledge is needed on this point, since pharmacological activity is likely to be better related to the drug concentration at the site of action than to the plasma concentration. Using a specific high-pressure liquid chromatographic (HPLC) separation method coupled with fluorimetric detection, we investigated DX concentrations in plasma, breast carcinoma, and some other surgically accessible tissues in seven patients with locally advanced breast cancer undergoing preoperative chemotherapy.

MATERIALS AND METHODS

Selection of Patients

Between March 1985 and April 1986, seven consecutive untreated patients with locally advanced (T3b-T4; N0-N3) breast cancer entered this study. All patients had a biopsy-proven diagnosis of breast carcinoma, life expectancy of at least 12 weeks, and performance status > 50 (Karnofsky score). Patients with inflammatory breast cancer (erythema, ridging of the skin, and high local temperature) were excluded. Eligibility criteria included: (*a*) age < 70 years; (*b*) adequate blood cell count (wbc count > 4000/mm³ and platelet count > 120,000/mm³); (*c*) bilirubin < 1.5 mg/dl and serum transaminases < 1.5 times normal; (*d*) serum creatinine < 1.5 mg/dl; and (*e*) no renal or hepatic metastases. Prior to entry, informed written consent was obtained from each patient. Subjects with history of congestive heart failure or serious hypertension were excluded.

Treatment Plan

DX for clinical use was supplied by Farmitalia-Carlo Erba in ampoules containing 10 mg. Table 1 describes the main clinical features in seven patients suffering from locally advanced breast cancer who received the first DX dose preoperatively. The drug was administered at the dose of 75 mg/m² in a 2-minute bolus injection. A simple or modified radical mastectomy was performed 48 hours later. Postoperative chemotherapy continued at the same dose every 3 weeks until a cumu-

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TABLE 1.—Clinical features of breast cancer patients

Patient No.	Performance status	Age (yrs)	Sex	Clinical stage
1	70	49	F	T4bN0M1
2	80	68	M	T3bN0M0
3	80	64	F	T4bN2M0
4	60	61	F	T4bN0M0
5	70	56	F	T4cN2M1
6	60	69	F	T4bN2M1
7	100	65	F	T4bN0M0

lative dose of 550 mg/m^2 or progressive disease or severe toxicity.

Sample Collection

A tumor excisional biopsy (500 mg) was taken 30 minutes and 24 hours after iv DX. All specimens consisted of conical excision; the skin and the deepest layer were removed to provide pathological analysis while the central section was utilized for DX determination. Within each patient subsequent biopsies were performed in the same quadrant, whenever possible in the first excision site. During mastectomy, performed 48 hours after DX injection, samples from neoplastic and unaffected breast tissue, small pectoralis muscle, any infiltrated muscle, and macroscopically metastatic and unaffected axillary lymph nodes were collected and frozen at −20°C. In Patient 5 with inoperable disease, only a third breast cancer biopsy was obtained. All these tissue samples were histologically verified.

Blood samples (3 ml) were taken through an indwelling cannula before drug injection and from the arm not receiving chemotherapy 5, 10, 15, 30, and 60 minutes and 2, 3, 4, 8, 12, 24, and 48 hours after. Samples were immediately put into heparinized tubes, spun down at 2000 rpm for 10 minutes, and the supernatant was frozen at −20°C. Samples of urine (10 ml) taken before and 24 and 48 hours after the first DX injection were stored at −20°C.

Histopathology

All tissue specimens were fixed in Bouin's solution and embedded in paraffin; thin sections (3–4 μm) were routinely stained with hematoxylin and eosin. The following microscopic features were evaluated for each case: histological grading (G) according to World Health Organization (WHO) criteria, mitotic index (MI), and the presence of tumoral necrosis. Mitoses were counted by scanning each section available (at least two sections) from tumor biopsies, tumor from the mastectomy specimen, and metastatic lymph nodes. Only distinct mitotic figures were recorded. The area of each microscopic field occupied by neoplastic cells (%) in relation to the amount of fibrosis was also recorded. For each section the number of microscopic fields counted ranged from 11 to 103 (mean, 43).

MI was defined as the ratio between the total number of mitoses and the number of entirely neoplastic microscopic fields, counted in the section with the most active areas.

A semiquantitative and qualitative analysis was made to assess the degree (0 to ++) and the characteristics of necrosis regarding the type of distribution and the cellular composition, respectively. An additional analysis was performed in blind by an independent pathologist, Dr. E. Pedrinis, Department of Pathology, Locarno, Switzerland.

Drug Assay

Plasma samples (0.5–1 ml) were extracted with 8 ml of isopropanol:chloroform (1:1) after addition of 100 μl of a solution containing daunorubicin as internal standard.

Tissue samples were homogenized in 10 volumes of water and processed as previously described (7). In brief, 100 μl of a solution containing daunorubicin as internal standard and 30 μl of 33% $AgNO_3$ were added to 0.5–1 ml of tissue homogenate. The samples were then extracted with 8 ml of isopropanol. The organic phase was evaporated under vacuum and dried; samples were then redissolved with 100 μl of methanol and injected into a Perkin Elmer series 3 B HPLC with a fluorescence detector (Perkin Elmer 650/10, Perkin Elmer, Norwalk, CT, USA) at an excitation wavelength of 475 nm and an emission wavelength of 580 nm.

Separation was achieved by an isocratic solvent system of acetonitrile:water:0.1 M phosphoric acid (32:42:26) at a flow rate of 1.2 ml/minute on a 25-cm "LiChrosorb" C-18 column (E. Merck, Darmstadt, Federal Republic of Germany). The sensitivity limit was 10 ng/g of tissue.

Pharmacokinetic analysis.—Plasma kinetic data were fitted using the following equation:

$$C_p = A\ e^{-\alpha t} + B\ e^{-\beta t} + C\ e^{-\gamma t}$$

where C_p is the plasma concentration at time t; A, B, and C are the intercepts on the ordinate at time zero, and represent the slopes of the respective exponential segment. T/2α, β, and γ were calculated as 0.693/α, 0.693/β, and 0.693/γ. Area under the curve (AUC) was calculated by the trapezoidal rule. Plasma clearance (C1) was calculated as dose/$AUC_{0\to\infty}$; volume of distribution (Vd) was calculated as Cl/γ.

RESULTS

Preoperative DX treatment was well tolerated; no patient experienced toxicity or wound-healing complications.

Table 2 summarizes some information on the microscopic findings of the same biopsies in which DX concentration was determined. The grading analysis of the different biopsies was generally consistent in each case; the MI decreased in all cases from 30 minutes to 24 hours after DX and further between 24 and 48 hours. The presence of necrosis appeared to increase 24 and 48 hours after drug treatment. Figure 1 shows the

TABLE 2.—Pathological parameters after DX treatment

Patient No.	Parameter*											
	Grading				MI				Necrosis†			
	1	2	3	LN	1	2	3	LN	1	2	3	LN
1	G2	G1	G1	G1	1.04	0.78	0.38	0.42	0	0	0	0
2	G1	G2	G2	G2	0.97	0.16	0	0.26	0	0	+	+
3	G3	G3	G3	G2	0.76	0.21	0.03	0.06	0	+	+	+
4	G2	G2	G3	G2	0.90	0.04	0	0.04	0	0	+	0
5	G1	G1	G2	ND	0.09	0	0	ND	0	0	0	ND
6	G3	G3	G3	G3	8.80	5.90	6.10	9.20	+	++	++	++
7	G3	G2	G2	G2	2.90	0.90	0.20	0.30	0	+	+	++

*1 = after 30 mins; 2 = after 24 hrs; 3 = after 48 hrs; and LN = metastatic lymph node after 48 hrs.

†Score: 0 = absent; + = moderate; ++ = high; and ND = not determined.

plasma DX profile in the seven cases investigated and table 3 summarizes the main pharmacokinetic parameters. A triexponential equation gave the best fit for plasma levels. The terminal half-life ranged between 20.5 and 55 hours, Vd between 387 and 884 L/m^2, and Cl between 129 and 328 ml/min/m^2. Urinary excretion of DX or doxorubicinol (DXol) amounted to 0.7%–9.8% and 0.6%–4.6% of the dose, respectively.

Table 4 shows tumor DX concentrations and the tumor/plasma DX concentration ratios 30 minutes, 24 hours, and 48 hours after drug injection. Already at 30 minutes DX tumor concentrations were much higher than in plasma. At subsequent times there were wider differences in drug concentrations in tumor than in plasma. Plasma drug concentrations decreased markedly from 30 minutes to 24 hours and further from 24 hours to 48 hours. In the tumor, DX concentrations declined from 30 minutes to 24 hours, except in Patient 2 in whom the drug concentration at 24 hours was higher than at 30 minutes. The tumor drug concentration, however, clearly fell less than in plasma; this was well indicated by the increase in the ratios of tumor/plasma concentrations. Tumor/plasma AUC ratios up to 48 hours ranged between 21 and 210.

Figure 2 shows DX concentrations at 48 hours in breast carcinoma and other surgically accessible tissues. Dx concentrations in normal breast were lower than (in Patients 1,4, and 6) or similar (Patient 7) to those in the tumor. DX levels were very low in fat and skin, slightly higher in muscle, and very high in normal or metastasized lymph nodes.

DXol was never detectable in the tissues examined when the sensitivity of the assay was 10 ng/g.

DISCUSSION

The antitumoral agent DX reached much higher concentrations in human breast carcinoma than in plasma. Tumor concentrations ranged between 3.8 and 19 μg/g (median, 8.4 μg/g) at 30 minutes; between 1.9 and 30.4

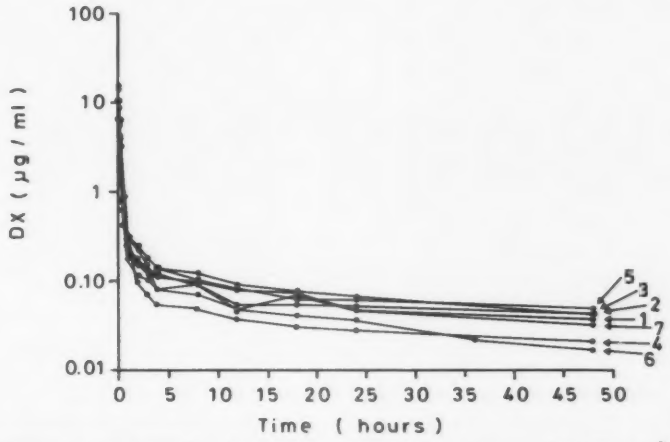

FIGURE 1.—DX plasma kinetic profiles in 7 breast cancer patients receiving 75-mg/m^2 iv doses of DX.

TABLE 3.—Plasma pharmacokinetic parameters and urinary excretion of DX in patients with breast cancer

Patient No.	T½ α (mins)	T½ β (mins)	T½ γ (hrs)	$AUC_{0-\infty}$ (μg/ml·min)	Vd (L/m^2)	Cl ($ml/min/m^2$)	Urinary excretion (% of the dose) DX	Urinary excretion (% of the dose) DX as DXol
1	3.7	47.2	26.9	362	484	207	1.1	0.6
2	4.6	44.5	55.0	496	721	151	2.1	1.2
3	4.9	40.1	28.0	477	382	157	4.1	1.4
4	3.8	48.3	38.1	280	884	268	1.6	0.6
5	3.9	89.2	51.7	581	578	129	0.7	0.7
6	4.9	70.2	20.5	228	583	328	9.8	4.6
7	5.5	113.9	42.8	462	602	162	1.6	1.4
Mean	4.5	64.8	37.6	412	605	200	3.0	1.5
± SE	0.25	10.5	4.9	48	61	27	1.2	0.53

μg/g (median, 2.86 μg/g) at 24 hours; and between 1.5 and 14.2 μg/g (median, 4.5 μg/g) 48 hours after iv injection of 75 mg/m^2 of DX. Tumor $AUC_{0-48\ h}$ values were 21–210 times the plasma $AUC_{0-48\ h}$ values.

The very high and long-lasting tumor DX concentrations are in line with previously reported data on DX distribution in tumor-bearing animals. For example, Broggini et al (6) reported that in Lewis lung carcinoma-bearing mice DX AUCs in plasma and tumor were respectively 366 μg/ml·min and 5345 μg/ml·min after an iv dose of 15 mg/kg.

As previously found in some animal tumors, in human breast cancer DX distribution also reaches an equilibrium many hours after injection, as indicated by the fact that the concentrations at 48 hours were even higher than at 24 hours (Patients 1, 5, and 7) or only slightly lower (Patients 3, 4, and 6). The prolonged retention of DX in the neoplastic tissue may be also partially due to the drug-induced necrosis with consequent less efficient blood drainage.

Not much information is available on DX levels in human breast cancer. Lee et al (8) investigated DX concentrations in different human tumors at a single, relatively early time point (between 1.5 and 5 hours after injection) using a thin-layer chromatographic fluorescence scanning technique. Their case list consisted mostly of liver tumors or sarcomas but two cases of breast carcinoma were included. In these two cases DX concentrations were 0.29 and 2.49 μg/g 2 hours after respective iv DX doses of 1.07 and 0.6 mg/kg. DX plasma levels in these two cases were not reported, thus making impossible any consideration on the tumor/plasma ratio, but if normalization of the dose is applied, the difference in DX tumor concentrations between the two cases would be 15-fold.

Therefore the results shown in the present study as well as the two cases reported by Lee et al (8) clearly indicate broad variability in DX distribution in breast carcinoma. Even though tumor AUC values should be taken with caution and used only as an indicative parameter since they were calculated on only 3 points for each patient, their variability is indeed much greater than for plasma AUC. For example tumor DX AUC in Patient 2 was 9.5 times greater than in Patient 4, whereas their plasma AUC values differed by < 2 times.

Though the number of cases was too small to permit any firm conclusion, DX concentration in breast carcinoma is apparently not directly related to the pathological features of the tumor. For example DX tumor concentrations were very similar in Patient 6 (the least

TABLE 4.—DX concentrations in breast tumor 30 mins, 24 hrs, and 48 hrs after iv drug

Patient No.	Time (30 mins) Tumor DX concentration (μg/g)	Time (30 mins) Tumor concentration/plasma concentration	Time (24 hrs) Tumor DX concentration (μg/g)	Time (24 hrs) Tumor concentration/plasma concentration	Time (48 hrs) Tumor DX concentration (μg/g)	Time (48 hrs) Tumor concentration/plasma concentration	Tumor AUC (μg/g·min)	Tumor AUC*/plasma AUC
1	5.32	12.8	2.86	62.8	4.52	125.9	11,160	40.1
2	11.95	14.6	30.36	583.8	14.17	337.4	62,069	210.2
3	19.02	21.7	10.40	157.6	10.12	246.8	35,800	948.0
4	3.81	5.0	1.86	69.0	1.54	74.6	6502	30.7
5	3.77	6.1	2.16	35.1	2.66	55.2	7707	21.1
6	9.58	22.8	3.90	111.4	3.64	227.5	15,075	75.1
7	8.42	11.1	2.86	62.1	4.62	149.0	13,464	38.8

*Tumor and plasma AUC were calculated using the trapezoidal rule up to 48 hrs.

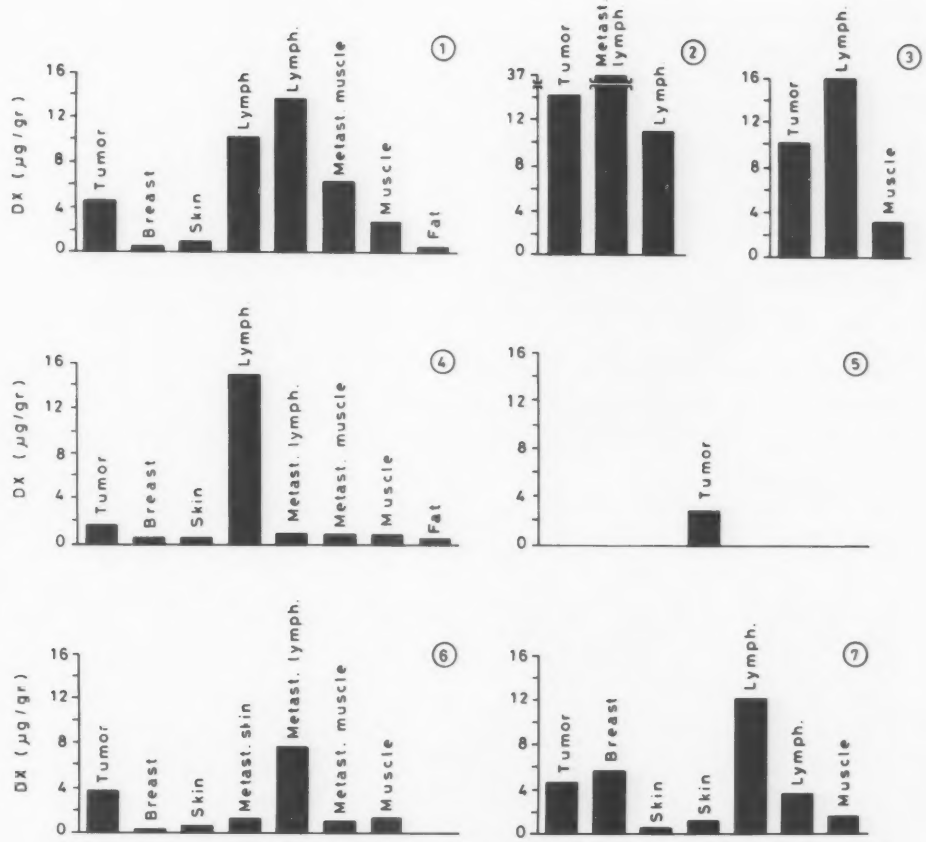

FIGURE 2.—DX concentrations in surgically accessible specimens obtained 48 hrs after drug injection (75 mg/m^2 iv) from 7 breast cancer patients.

differentiated tumor, with areas of necrosis) and in Patient 1 (a well-differentiated tumor without any evidence of necrosis). Therefore it appears that microscopic morphological analysis provides no worthwhile information on the tumoral features underlying low or high DX uptake by breast carcinoma. It is known that intracellular DX concentration is crucial for drug cytotoxicity (9). It has been recently reported that in some cell lines resistant to DX (and to a number of other structurally related and unrelated compounds) intracellular DX concentrations were much lower than in sensitive cell lines and the low DX retention appeared to be associated with the presence of some membrane glycoproteins of molecular weight 170 Kd (9–11). Therefore the variable DX concentrations in breast carcinoma of patients receiving the same DX dose could perhaps be related to the biochemical characteristics of the tumor cells (eg, membrane glycoproteins) and might account for the different drug efficacy in different patients. A very recent report has also suggested that DX tumor concentration may be an important determinant of drug efficacy since biopsies (obtained 27 ± 16 mins after injection of 25 mg/m^2 of DX) of breast and colon cancer contained an average of 819 and 176 μg/g, respectively, the two tumors being responsive to DX in 39% and 9% of the cases, respectively (12).

Obviously we could not attempt any correlation between the tumor drug levels and clinical response because of the small number of patients investigated, but further studies focused on this point in a larger population should be planned.

As also reported by Lee et al (8) DX levels were low in human skin, fat, and muscle and very high in lymph nodes. The latter finding may be relevant since nodes are often a site of metastases of breast carcinomas and other human malignancies.

We did not detect DXol in the tissues analyzed when the limit of the analytical assay was 10 ng/g. Therefore, even if this metabolite were present in the tumor, considering that it is not more cytotoxic than DX (13), we

can reasonably conclude that its antitumoral role is likely to be negligible in breast cancer.

As previously reported (1,2), renal excretion is not a major route of elimination of DX since 48-hr urinary excretion of unchanged drug or DXol did not exceed 10% of the dose.

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Testing New Drugs in Untreated Small Cell Lung Cancer May Prejudice the Results of Standard Treatment: A Phase II Study of Oral Idarubicin in Extensive Disease[1,2]

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We have evaluated the orally active anthracycline idarubicin at a dose of 40 mg/m² in divided doses over 24 hours in 21 previously untreated patients with extensive-stage small cell carcinoma of the lung (SCCL). Subsequent iv therapy was cyclophosphamide (1000 mg/m²), vincristine (1 mg/m²), and etoposide (120 mg/m² iv on Day 1 and 250 mg/m² orally in divided doses on Day 2; CVE) in patients who failed to respond to idarubicin and in relapsed patients. Three (14%) of 21 patients treated with idarubicin responded, with two complete responses (CR). Patients failing to respond promptly and those who relapsed were treated with CVE. Seven patients did not receive CVE for the following reasons: early death, four patients; early CNS disease, two; and refusal, one. Fourteen patients received CVE. Of 12 patients failing to respond to idarubicin, eight progressed on CVE, three achieved partial response (PR), and one achieved CR. Two idarubicin responders who received CVE achieved PR and CR. The median survival of all 21 patients was 6 months. For those with World Health Organization performance scores of 0 or 1 the median survival was 6.2 months and for the rest it was 2.6 months. Although CVE chemotherapy was instituted promptly in patients not responding to idarubicin, these results seem inferior to those previously seen in our center with standard treatment from the start. We believe that patients with extensive SCCL often require a rapid and more guaranteed response to their first treatment. [Cancer Treat Rep 71:1227-1230, 1987]

The results of standard chemotherapy in extensive small cell lung cancer (SCCL) are poor, with virtually no long-term survivors (1). However, a number of chemotherapeutic agents in combination can provide effective short-term palliation and extension of survival. Conventionally, new drugs are tested in patients who have failed on standard treatment and thus may have multidrug-resistant tumors. It is quite possible that potentially valuable agents will not be identified in this setting. Thus, a number of investigators have tested new drugs in previously untreated patients (2,3). This approach allows for a realistic assessment of the drug's activity, but is an ethical approach only if those patients who do *not* benefit in terms of response to the new treatment do not suffer from having received it. This problem has not been addressed in previous reports of new drugs, or new analogues of active existing drugs, in untreated patients.

Idarubicin is a derivative of daunorubicin which lacks the methoxy group at position 4 in the aglycone ring. As well as being an active antitumor agent in murine leukemia and solid tumor models (4,5), it has been shown to be active when administered orally (6). In addition, it appears to be significantly less cardiotoxic than daunorubicin or doxorubicin in animal studies (7). Doxorubicin is one of the most active agents in SCCL (1), but must be administered iv. Apart from this inconvenience, extravasation reactions to doxorubicin can be very severe, and the prospect of a less cardiotoxic, orally active agent in SCCL prompted this evaluation in previously untreated patients with extensive disease. Patients who failed to show a good response to idarubicin at any stage were switched to iv therapy with three standard, nonanthracycline agents with proven activity against SCCL. Thus, we also report the results of treatment with the standard, second-line iv regimen and the overall survival of the study patients.

PATIENTS AND METHODS

Twenty-one patients (median age, 64 years old; range, 43-76) with histologically proven SCCL who had not

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[2] Idarubicin was supplied by Farmitalia Carlo Erba, United Kingdom.

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TABLE 1.—Sites of metastases

Site of metastases	No. of patients
Liver	8
Bone	5
Sc	2
Bilateral chest	2
Chest wall	1
Retroperitoneum	1
Liver and bone	1
Bone and retroperitoneum	1

previously received chemotherapy or radiotherapy were entered in this study. All patients had evaluable, extensive disease based on results of clinical examination, chest x-ray, routine blood tests, isotopic scans of bone and liver, and, where appropriate, examination of marrow aspirate and computerized tomographic scans of chest or abdomen. The sites of metastases and performance status categories are shown in tables 1 and 2. Patients were excluded if there was evidence of (*a*) bone marrow hypoplasia (peripheral wbc count $\leq 3.5 \times 10^9$/L or platelet count $\leq 100 \times 10^9$/L unless due to marrow involvement by tumor); (*b*) renal insufficiency; (*c*) hyperbilirubinemia; (*d*) CNS involvement; or (*e*) heart disease. All patients gave signed, informed consent to participation in the study, which was approved by the Central Birmingham Health District Research Ethical Committee.

Patients were treated with idarubicin at 40 mg/m^2 given orally in divided doses over 24 hours. This was repeated every 21 days provided peripheral blood cell counts had recovered sufficiently. Blood cell counts were also performed on Days 10 and 14 of the first two courses of treatment, which provided information on patient compliance and drug absorption and allowed for dose escalation. In those patients not encountering myelosuppression during the first-course nadir period, the dose of subsequent courses was increased by 5 mg/m^2 until myelosuppression was seen. A maximum of six courses of idarubicin was given to responding patients. At this time tests of functions known to have been abnormal before treatment were repeated, but this did not include repeat bronchoscopy. Patients were classified as having achieved complete response (CR) if all pretreatment disease-related abnormalities had resolved.

Treatment was changed to conventional combination chemotherapy (cyclophosphamide at 1000 mg/m^2; vincristine at 1 mg/m^2; and etoposide at 120/m^2 iv on Day 1 and 250 mg/m^2 orally on Day 2 [CVE]; treatment was given every 21 days for a maximum of six courses) in the following circumstances: (*a*) objective disease progression at any stage; (*b*) failure to achieve symptomatic improvement at any stage; (*c*) stable disease after a maximum of two courses of idarubicin (6 weeks), irrespective of symptomatic improvement; and (*d*) achievement of partial response (PR) or CR on idarubicin at the time of relapse or disease progression.

TABLE 2.—WHO performance scores at start of idarubicin therapy

WHO performance score	No. of patients
0	1
1	10
2	7
3	2
4	1

RESULTS

All 21 patients were evaluable for response to idarubicin. Sixteen of the 21 patients received only one or two courses. There were two CRs lasting 5.5 and 8.5 months and one PR for an overall response rate of 14% (95% confidence interval, 0%–29%). The two patients who achieved CR received six courses of idarubicin, but the patient who achieved PR developed CNS disease after two courses and was withdrawn from further chemotherapy. Three patients received three, four, and five courses of idarubicin since they showed minor response. Thirteen patients had a wbc count $< 3.5 \times 10^9$/L and/or a platelet count $< 100 \times 10^9$/L at the starting dose of 40 mg/m^2. With dose escalation a total of 15 patients experienced midcycle myelosuppression during one or more courses of idarubicin. The six patients in whom a nadir of this level was not observed were those who showed progressive disease during the first course and therefore either were switched to CVE or died before that was possible. The principal side effects of idarubicin were nausea, vomiting, and alopecia.

Seven patients did not receive CVE for the following reasons: four deteriorated rapidly and died of progressive disease during the first 6 weeks; two developed CNS metastases during the first 6 weeks and were withdrawn from further chemotherapy; and one refused CVE. Fourteen patients did receive CVE: in nine this was after one or two courses of idarubicin; in three (the minor idarubicin responders) it was after three, four, or five courses; and in two (the CRs achieved from idarubicin) it was at the time of disease progression. The 12 patients who did not respond to idarubicin responded to CVE as follows: one had CR; three had PR; and eight had disease progression. The two patients who had CR with idarubicin achieved PR and second CR with CVE. The overall response rate to CVE was 43% (95% confidence interval, 20%–72%).

The median duration of survival for the group as a whole was 182 days (6 months). The survival curve is shown in figure 1. Survival in patients with good performance status [World Health Organization (WHO) 0 or 1)] was significantly better than in those with poor performance status ($P < 0.02$) (fig 2). The median survival for those patients with good performance status was 190 days (6.2 months) compared to 80 days (2.6 months) for the rest.

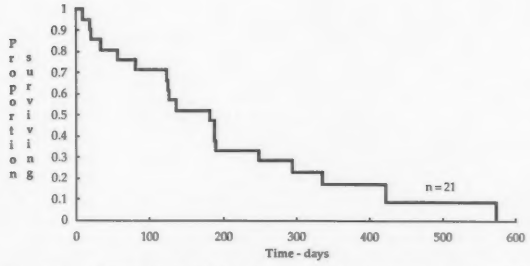

FIGURE 1.—Overall survival of 21 patients with extensive SCCL treated initially with idarubicin.

DISCUSSION

Idarubicin has also been evaluated in advanced nonpretreated SCCL by Milroy et al (8). Using a slightly higher dose (50 mg/m^2 in divided doses) orally over 24 hours, they observed no PRs or CRs among 14 evaluable patients. However, nine patients had minor improvement. In the present series, we observed three objective responses and three minor responses. This drug, when given as an oral preparation over 24 hours, has only modest activity in SCCL. Starting with 40 mg/m^2, we recorded midcycle myelosuppression in 13 of 21 patients and with subsequent dose escalation this increased to 15 patients. However, there remains doubt about compliance and absorption in six patients in whom myelosuppression was not observed. This is a potential problem with all oral chemotherapy. As for toxicity, our experience is similar to the reported series, with mild to moderate nausea and vomiting and alopecia.

Few details of subsequent treatment are given in the report by Milroy et al (8) or in most other series where previously untreated patients with SCCL are given new agents or analogs. However, Milroy does state that only four of their 14 patients were able to receive subsequent combination chemotherapy. Their patients all had Eastern Cooperative Oncology Group performance status of 2 or better and received a median of three courses of idarubicin. The inference is that a majority of their patients deteriorated while receiving idarubicin to the extent that conventional treatment became impossible. The median survival of those patients having idarubicin as the sole treatment was only 3 months.

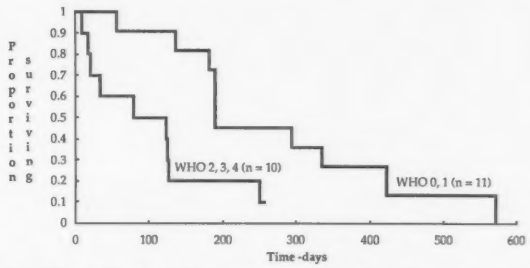

FIGURE 2.—Survival of 11 patients with good performance status (WHO 0 or 1) compared to 10 patients with poor performance status (WHO 2, 3, or 4); $P < 0.02$.

Survival in extensive-stage SCCL is poor, with a median in large series of around 8 months (9). A small proportion of patients, even with hepatic metastases, may survive 2 years with conventional iv combination chemotherapy (10). Consequently, these patients are suitable for new-drug evaluation only if it can be conducted without prejudicing standard treatment. The present study was specifically designed to minimize this risk, in that only those patients showing an objective response continued receiving idarubicin after two courses. Furthermore, evidence of disease progression at any stage, even after a single course of idarubicin, prompted immediate withdrawal from experimental therapy and conventional treatment was started. However, despite strict adherence to this policy, one-third of patients never received combination chemotherapy. Eight of the 14 patients who did receive CVE failed to respond, and the overall survival of patients in this study was shorter than one would expect for a similar group of patients with extensive-stage SCCL treated with combination chemotherapy de novo.

The response rate to CVE was only 43%. The numbers are small and the confidence interval is wide. Twentyman et al (11) have shown that growing human SCCL cell lines in the presence of doxorubicin can induce resistance, not only to the anthracycline but also to vincristine and etoposide (11). It is possible that similar phenomena occur in patients, particularly with a drug like idarubicin which is extensively metabolized to an active compound (4-demethoxydaunorubicinol) with a long half-life (12).

There is a fundamental difficulty in evaluating new drugs in tumors for which there are a number of standard active agents that are used first. It is well known that previously treated patients have tumors which are much less chemosensitive than untreated tumors. Bork et al (2) demonstrated in their study of teniposide in SCCL that potentially valuable drugs may be missed in this setting. The analog studied by Bork et al (2) proved to be highly active and their overall results were not prejudiced by exposure to teniposide. The same may be true of the study by Smith et al (3) using carboplatin. Again this proved to be an extremely active agent. These authors argue that there is little likelihood of long-term survival in these groups, regardless of an initial response to conventional chemotherapy, and that they are suitable for investigational drugs. We believe that these patients may not be appropriate for new-drug evaluation, since if the agent under study proves to be relatively ineffective, then these patients do not have time and may not be able for other reasons to achieve the worthwhile symptomatic response combination chemotherapy can offer for the majority of patients with extensive SCCL.

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Pharmacokinetics of Carboplatin After Iv Administration[1,2]

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Pharmacokinetics of the cisplatin analog carboplatin were studied in ovarian cancer patients who received short-term iv infusions of 290–370 mg/m². Platinum (Pt) was determined by atomic absorption spectrometry in plasma ultrafiltrate up to 24 hours and in plasma and urine up to 5 days following infusion. Carboplatin was determined in plasma ultrafiltrate and in urine by high-performance liquid chromatography with electrochemical detection. The final half-life of total Pt in plasma was 5.8 ± 1.6 days. Pharmacokinetics of carboplatin and ultrafilterable Pt (free Pt) were similar with respect to α-half-life (16 ± 6 and 23 ± 8 mins), β-half-life (118 ± 15 and 120 ± 11 mins), area under curve/dose (18 ± 5 and 17 ± 4 min/m²/L), total-body clearance (101 ± 21 and 107 ± 19 ml/min), and volume of distribution V_{ss} (9.9 ± 1.3 and 10.0 ± 1.4 L/m²). After 6 hours the cumulative urinary excretion of carboplatin and Pt was 41% ± 14% and 68% ± 7% of the dose, respectively. After 5 days the cumulative urinary excretion of Pt was 84% ± 6%. Renal and metabolic clearances of free Pt from plasma were 81 ± 17 and 26 ± 11 ml/minute, respectively. The first-order rate constant for metabolic elimination of free Pt ($K_M = CL_M/V_{ss}$) was $1.5 \times 10^{-3} \pm 0.6 \times 10^{-3}$ min^{-1}, which is ten times lower than the value calculated from literature data for cisplatin ($15 \times 10^{-3} \pm 1 \times 10^{-3}$ min^{-1}). This means that the overall in vivo reactivity of carboplatin is ten times lower than that of cisplatin. [Cancer Treat Rep 71:1231-1237, 1987]

Carboplatin, an analog of the antitumor drug cisplatin, has shown appreciable activity against ovarian cancer (1,2). It is less nephrotoxic, neurotoxic, and emetogenic than cisplatin (1,3). Carboplatin-induced myelosuppression is dose-limiting and the maximum tolerated dose (MTD) has been estimated at 300–500 mg/m² in previous phase I trials (1,3). Because of its favorable toxicity profile, carboplatin was and still is evaluated extensively in clinical studies exploring its antitumor activity in comparison with cisplatin (3–7). Comparison with cisplatin was also made in preclinical studies (8–10) and in in vitro studies investigating cytotoxicity and interaction with DNA (11,12). The latter studies revealed that equal amounts of platinum (Pt)-DNA binding induced by different amounts of cisplatin and carboplatin produced equal cytotoxicity.

Pharmacokinetics of cisplatin have been studied extensively (13–17). Comparison of the pharmacokinetics of carboplatin with those of cisplatin will contribute to a better understanding of its therapeutic and toxic effects. So far, pharmacokinetic studies were performed during phase I trials of carboplatin revealing linear pharmacokinetics of Pt (18–22). In one study (21) the original drug was also measured but the limited time interval (4 hours after administration) precluded a proper calculation of pharmacokinetic parameters. For the same reason the final elimination phase of total Pt in plasma has not yet been determined. Patients in the mentioned reports received a wide range of doses and their renal function varied widely. Therefore, the purpose of this study was to assess the pharmacokinetics of carboplatin, ultrafilterable Pt (free Pt), and total Pt in plasma, rbcs, and urine up to 5 days after a short-term iv infusion of carboplatin in patients with advanced ovarian cancer who had normal renal function.

PATIENTS AND METHODS

Patients

Seven patients with advanced ovarian carcinoma were entered in the study. All patients but one received carboplatin as part of a combination therapy including doxorubicin (given on the same day as carboplatin), hexamethylmelamine, and cyclophosphamide (both given orally from Day 15 to Day 28). Two patients (Nos. 1 and 2; table 1) had never received Pt compounds. All

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patients had normal kidney function (creatinine clearance > 60 ml/minute) except one (No. 3; creatinine clearance, 52 ml/minute), and all had normal liver function. Two patients (Nos. 3 and 4) had malignant effusion. The median age was 55 years (range, 45–66). Vials of lyophilized carboplatin were obtained from Bristol-Myers (Weesp, The Netherlands). The contents were reconstituted with water for injections to obtain a concentration of 10 mg/ml. The appropriate amount was diluted with 5% glucose to 250 ml. This volume was administered with a constant infusion rate in 30–37 minutes (dose range, 290–370 mg/m^2).

Sampling

Blood samples (5 ml) were collected in heparinized tubes prior to, half-way through, and at the end of infusion, as well as at 10, 20, 30, 60, 90, 120, 150, 180, 240, 300, 360, and 540 minutes and 24, 48, 72, 96, and 120 hours following the infusion. Samples were processed immediately after collection. Blood was centrifuged and two portions of 1 ml of plasma were ultrafiltrated by MPS-1 systems provided with YMT filters (cutoff, 30,000 daltons) (Amicon, Oosterhout, The Netherlands) (23). The remaining plasma (for total Pt analysis) and both plasma ultrafiltrate samples (for free Pt and carboplatin analyses, respectively) were immediately frozen on dry ice. Rbcs were washed twice with an equal volume of normal saline at room temperature. Urine was successively collected up to 2, 4, 6, 12, 24, 48, 72, 96, and 120 hours after the start of infusion. Small portions of the urine collected over the first three time intervals were frozen immediately. All samples were stored at −25°C and thawed just before analysis.

Analysis

Pt concentrations in plasma (total Pt), plasma ultrafiltrate (free Pt), rbcs, and urine were determined by atomic absorption spectrometry according to the method described earlier (17). High-performance liquid chromatography (HPLC) with differential pulse polarographic detection (24) was used to determine the original drug in plasma ultrafiltrate and urine. The limits of detection by this method were 0.1 and 1 μM and the coefficients of variation were 1.5% and 4.7% in plasma ultrafiltrate and urine, respectively.

Stability of Carboplatin

Plasma ultrafiltrate and urine of healthy volunteers were spiked with aqueous carboplatin solutions of 5 mM to obtain a final concentration of 100 μM.

Three portions of spiked plasma ultrafiltrate and urine were incubated at room temperature. Two samples of 0.15 ml were withdrawn from each portion at regular time intervals over an incubation period of 10 days. Carboplatin was analyzed in the samples immediately after withdrawal.

Two other portions of spiked plasma ultrafiltrate and urine were analyzed for carboplatin, divided in five aliquots of 0.4 ml, and frozen at −25°C. Aliquots were thawed and analyzed in duplicate at regular time intervals over a period of 21 weeks. This test was repeated over a storage period of 40 weeks.

Stability of carboplatin in plasma ultrafiltrate was also tested at 37°C by the daily analysis of two samples over a period of 6 days.

Two patients (Nos. 6 and 7) were treated with carboplatin in combination with ifosfamide and mesna. The administration of both ifosfamide and mesna was started at 4 hours after the administration of carboplatin. Therefore, the stability of carboplatin was also tested in aqueous solutions of 50 μM carboplatin in the presence of 0.2 mM dimesna (2,2′-dithiodiethanesulfonate disodium, supplied by Dr. J. Pohl, Asta Werke, Bielefeld, Federal Republic of Germany) at 37°C for 24 hours. The concentrations used were representative of those expected in plasma (25).

Pharmacokinetic Data Analysis

Postinfusion plasma concentration vs time curves were fitted by the computer program NONLIN (26) to a polyexponential equation, $C_p = \sum_{i=1}^{n} Y_i \cdot \exp,-\lambda_i t$. Each coefficient Y_i was corrected for the infusion time T to C_i (27) by: $C_i = (-\lambda_i T Y_i)/(\exp(-\lambda_i T) - 1)$. The area-under-the-plasma-concentration-versus-time curve (AUC = $\sum_{i=1}^{n} C_i/\lambda_i$), the area-under-the-first-moment curve (AUMC = $\sum_{i=1}^{n} C_i/\lambda_i^2$), and the total-body clearance (CL = D/AUC, D = dose) were calculated from the obtained coefficients and exponents (27,28). Renal clearance (CL_R) was defined as the cumulative urinary excretion (CUE) divided by the AUC, both measured from the start of infusion up to 6 hours after the end of infusion. The AUC (0–6 hours) was calculated by means of the linear trapezoidal rule. The metabolic clearance (CL_M) was calculated for carboplatin and free Pt from $CL_M = CL - CL_R$. The half-life of total Pt over Days 1–5 was separately determined by the least-squares method.

The volume of distribution at steady-state (V_{ss}) of total Pt was calculated as $V_{ss} = D/AUC \times AUMC/AUC$ (27,28). V_{ss}s of carboplatin and free Pt were calculated according to Collier (29) by $V_{ss} = D[f_1 \cdot MRT_1 + f_2 \cdot MRT_2]/AUC$, in which MRT_1 (= AUMC/AUC) and MRT_2 ($= 1/\lambda_1 + 1/\lambda_2$) (29) are the mean residence times (MRT) in the central and peripheral compartments, respectively, and f_1 and f_2 represent the fractions of the administered dose eliminated from each compartment. These fractions were estimated from CL_R and CL_M. CL_R only takes place from the central compartment, while CL_M takes place from the central ($CL_{M,1}$) and peripheral

($CL_{M,2}$) compartments. $CL_{M,1}$ is thought to be due to the same processes as occur in plasma in vitro, ie, protein binding and molecular degradation of carboplatin. Therefore, $CL_{M,1} = k_{in\ vitro} \times V_c$, in which $k_{in\ vitro}$ represents the in vitro elimination rate constant from plasma of carboplatin and free Pt, respectively, and V_c [= D/> C_i (28)] represents the volume of distribution of the central compartment. This means that clearance from the central compartment $CL_1 = CL_R + CL_{M,1}$ and clearance from the peripheral compartment $CL_2 = CL_M - CL_{M,1}$. Thus: $f_1 = CL_1/CL$ and $f_2 = CL_2/CL$. The first-order rate constant of metabolic elimination K_M, representing the overall reactivity of the drug towards body constituents (plasma as well as tissue components), was calculated by analogy with the overall first-order rate constant of elimination at steady-state $K_{ss} = CL/V_{ss}$ (30). Thus: $K_M = CL_M/V_{ss}$.

RESULTS

Stability of Carboplatin

At room temperature carboplatin (100 μM) degraded with half-lives of 19.9 and 6.7 days in plasma ultrafiltrate and urine, respectively. This means that no significant loss of carboplatin will occur when sample preparation takes place immediately after withdrawal. Half-lives of carboplatin at −25°C (storage conditions) in plasma ultrafiltrate were 28.6 and 28.0 weeks on two occasions. However, half-lives in urine varied considerably (212 and 32 weeks). These data indicate that samples can be analyzed within a few weeks after collection. The mean pseudo first-order degradation rate constant in plasma ultrafiltrate at −25°C ($k_{obs} = 0.0245\ week^{-1}$) was used to correct all carboplatin concentrations in plasma ultrafiltrate for degradation during storage. The appreciable difference in stability in urine was probably caused by differences in urine composition. Such differences have also been observed at 37°C (21). Therefore, no correction could be made for degradation in urine.

A half-life of 98 hours was observed for carboplatin in plasma ultrafiltrate at 37°C. During the 6 days of incubation carboplatin never reached a constant concentration, as reported elsewhere (31). No degradation of carboplatin was observed during the 24-hour incubation with dimesna. Thus, chemical interaction in vivo might be excluded. Aqueous stock solutions of carboplatin (1 and 0.1 mM) stored at ambient temperature protected from light did not show any decrease in concentration up to at least 1 month.

Clinical Pharmacokinetics

Concentration versus time curves of carboplatin and free Pt in plasma ultrafiltrate and total Pt in plasma as well as Pt in rbcs from a representative patient receiving a short-term infusion of carboplatin are shown in figure 1. No secondary peaks in any of these curves were noticed. Concentration-time curves of carboplatin and free Pt were quite similar in all patients for the first 9 hours. Thereafter, both curves diverged; ie, the concentration-time curve of carboplatin showed a faster drop than that of free Pt. Carboplatin and free Pt could be measured in plasma ultrafiltrate for at least 9 hours.

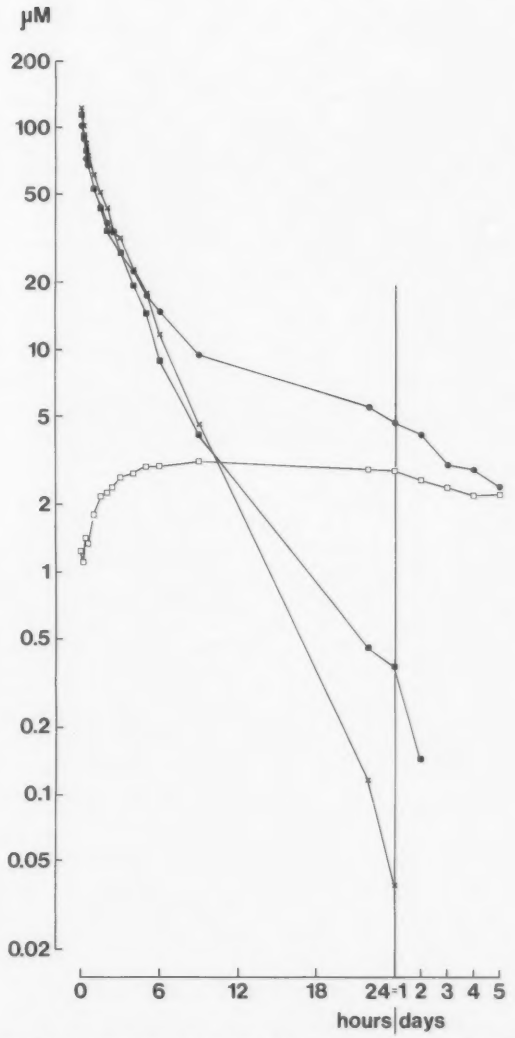

FIGURE 1.—Semilogarithmic plots of the concentrations of carboplatin (x), free Pt (■), and total Pt (●) in plasma and Pt in rbcs (□) vs time in Patient 4, who received 350 mg/m² over 37 mins.

Peak concentrations and basic pharmacokinetic parameters of individual patients as calculated by non-weighted NONLIN analysis are shown in table 1. Carboplatin and free Pt concentration-time curves were best fitted by two exponential equations. In contrast, all total Pt concentration-time curves declined with three exponential terms. The coefficient of determination r^2 (32) was > 0.996 for all curves.

TABLE 1.—Basic pharmacokinetic data of carboplatin in 7 patients as calculated by NONLIN

Parameter*	Patient No.— 1	2	3	4	5	6	7
Dose							
mg/m^2	367	343	290	352	349	301	299
μmols	1456	1617	1213	1415	1429	1348	1348
Carboplatin							
C_{peak} (μM)	129	102	140	123	125	84	90
C_1 (μM)	111	39	100	117	96	57	48
λ_1 ($\times 10^{-3}$ min^{-1})	31.3	56.2	35.9	66.3	50.2	64.1	27.1
C_2 (μM)	74	92	96	90	84	71	64
λ_2 ($\times 10^{-3}$ min^{-1})	6.59	6.50	5.19	5.27	5.11	6.62	6.43
Free Pt							
C_{peak} (μM)	134	109	124	116	110	84	90
C_1 (μM)	118	43	71	117	72	50	58
λ_1 ($\times 10^{-3}$ min^{-1})	27.4	23.3	25.5	60.1	45.6	22.7	37.9
C_2 (μM)	65	84	85	78	78	51	61
λ_2 ($\times 10^{-3}$ min^{-1})	6.50	6.29	5.29	5.32	5.28	5.56	6.39
Total Pt							
C_{peak} (μM)	125	113	128	103	106	90	93
C_1 (μM)	106	55	76	80	92	48	56
λ_1 ($\times 10^{-3}$ min^{-1})	20.3	22.8	29.2	46.7	82.9	49.9	22.9
C_2 (μM)	49	75	74	65	77	70	54
λ_2 ($\times 10^{-3}$ min^{-1})	5.80	6.66	5.40	5.17	6.65	7.00	5.65
C_3 (μM)	4.6	4.6	12.5	6.1	7.1	5.6	4.5
λ_3 ($\times 10^{-4}$ min^{-1})	0.69	0.84	0.99	1.31	1.62	1.38	0.75

*C_{peak} = observed peak concentration. Coefficients (C_1, C_2, and C_3) were corrected for the infusion time.

Mean values of common pharmacokinetic parameters are shown in table 2. Apart from the CUE, all parameters for carboplatin and free Pt were essentially the same. Initial half-lives of total Pt were comparable to those of carboplatin and free Pt. A long terminal half-life of 5.8 ± 1.6 days was separately calculated for total Pt over the discrete time interval of Days 1–5 by means of the least-squares method. This value was somewhat higher than the NONLIN value listed in table 2. The long terminal half-life resulted in high AUC values. Total-body clearance was much lower for total Pt than for free Pt and carboplatin. The high value of V_{ss} for total Pt indicates that an appreciable amount of Pt is bound to the tissues. Sixty percent of the Pt excreted in urine over the first 6 hours after administration consisted of carboplatin.

Table 3 shows the pharmacokinetic parameters related to the clearance of carboplatin in comparison to cisplatin. Total-body clearance of free Pt and carboplatin were about the same. The CL_R of free Pt was comparable with the mean creatinine clearance of 73 ± 18 ml/minute as measured the day before the start of therapy, indicating that CL_R will principally take place by glomerular filtration, as has been observed before (21,22). CL_R was lower for carboplatin than for free Pt, due to metabolic conversions of carboplatin. The overall CL_M of carboplatin was about twice that of free Pt. The CL_M of carboplatin and free Pt from the central compartment as estimated from their in vitro disappearance rates in plasma [0.0004 min^{-1} (21) and 0.00024

TABLE 2.—Pharmacokinetic parameters obtained in 7 patients after an iv infusion of carboplatin given over 30 mins*

Parameter	Carboplatin	Free Pt	Total Pt
$t_{½}\alpha$ (mins)	16 ± 6	23 ± 8	22 ± 10
$t_{½}\beta$ (mins)	118 ± 15	120 ± 11	116 ± 14
$t_{½}\gamma$ (days)	—	—	4.9 ± 1.5
AUC/D (min · m^2/L)	18 ± 5	17 ± 4	83 ± 32
CL (ml/min · m^2)	58 ± 12	61 ± 11	13 ± 3
V_{ss} (L/m^2)	9.9 ± 1.3	10.0 ± 1.4	103 ± 32
CUE (0–6 hrs)† %D	41 ± 14	68 ± 7	68 ± 7
CUE (0–24 hrs)† %D	—	77 ± 5	77 ± 5
CUE (0–5 days)‡ %D	—	84 ± 6	84 ± 6

*Values = mean ± SD.
†n = 5.
‡n = 3.

TABLE 3.—Pharmacokinetic parameters related to the clearance of carboplatin in comparison to cisplatin*

Parameter	Carboplatin: Carboplatin	Carboplatin: Free Pt	Cisplatin (free Pt)†
CL (ml/min)	101 ± 21	107 ± 19	354 ± 33
CL_R (ml/min)	44 ± 16	81 ± 17	74 ± 29
CL_M (ml/min)	57 ± 24	26 ± 11	281 ± 19
V_{ss} (L)	17 ± 2	17 ± 2	19 ± 2
K_M ($\times 10^{-3}$ min^{-1})	3.3 ± 1	1.5 ± 0.6	15 ± 1

*Values = mean ± SD. All values are normalized to 1.73 m^2 of body surface area.
†Derived from data of Vermorken et al (16,17); n = 3.

(23) min^{-1}] and V_c (9 ± 3 and 10 ± 3 L) were 3.6 and 2.4 ml/minute, respectively. With these values for $CL_{M,1}$, f_1 and f_2 were calculated to be 0.47 and 0.53 for carboplatin and 0.78 and 0.22 for free Pt, respectively. These f values were used to calculate the V_{ss} of carboplatin and free Pt (table 3).

For comparison, pharmacokinetic data of free Pt originating from cisplatin, as calculated from the data of Vermorken et al (16,17), are shown in table 3. Total-body clearance of free Pt originating from cisplatin was much higher than that from carboplatin. The CL_R of free Pt from cisplatin, as calculated from the AUCs and CUEs over the first hour after starting the short-term (7 minutes) infusions ($n = 3$), were similar to that of carboplatin. The overall CL_M of free Pt from cisplatin was much higher than that from carboplatin. The CL_M of free Pt from the central compartment was 71 ml/minute as calculated from the in vitro plasma degradation rate constant of 0.0068 min^{-1} (23) and a mean V_c of 10.5 L. Consequently, f_1 and f_2 were 0.41 and 0.59, respectively. The resulting V_{ss} of free Pt from cisplatin was similar to that from carboplatin. Finally, the metabolic rate constant K_M was much higher for free Pt originating from cisplatin than from carboplatin, indicating that in vivo carboplatin is a more stable compound than cisplatin.

Pt was taken up in rbcs and 66% ± 8% of the maximum concentration was reached after 60 minutes. Maximum levels of 2.5 ± 0.4 μM were reached at about 6 hours following infusion (median, 360 minutes; range, 240–540). At that time free Pt levels in plasma were still about three times higher than in rbcs. At the maximum concentration about 0.4% of the dose was present in rbcs. The Pt concentration in rbcs decayed monoexponentially, with a half-life of 12 ± 1 day as measured over Days 1–5.

DISCUSSION

The increasing clinical use of carboplatin as an alternative for cisplatin warrants detailed pharmacokinetic investigation. This implies determination of the original Pt complex and Pt concentrations in plasma for a period of time long enough to estimate the final elimination rate constants. Our sensitive and selective HPLC method (24) was capable of determining carboplatin for at least six final half-life times. Total Pt in plasma was determined up to 5 days following infusion, allowing a reliable fit of a triexponential equation for the concentration-time curves.

The concentration-time curves of total Pt in plasma coincided with those of free Pt and carboplatin for about 6 hours after administration, indicating that, in contrast to cisplatin, carboplatin binds only slowly to plasma proteins. This is in accordance with earlier reports (21) and in vitro observations (23,31,33). The slow protein binding is also reflected by a high cumulative urinary excretion of Pt, being 68% of the dose over the first 6 hours, compared to 24% of the dose for cisplatin over the same time interval (16).

No signs of enterohepatic recycling, as described for cisplatin (16,17), were observed. Biliary excretion of Pt may be lower after carboplatin than after cisplatin, as was also observed in rats (34), although in dogs the amount of Pt involved in enterohepatic recycling was higher after carboplatin than after the administration of cisplatin, iproplatin, spiroplatin, or JM-40 (35). Also, one clinical study reported secondary peaks in total and free Pt concentration–time curves (19). No definite conclusion can be drawn from these contradictory observations.

Total Pt concentrations in plasma at 24 hours after administration of 350 mg/m^2 of carboplatin (6 ± 3 μM) were similar to those obtained after 100 mg/m^2 of cisplatin [8 μM (16,17)]. Furthermore, the total amount of Pt present in the body after 24 hours, as calculated from the CUE over 0–24 hours, was 23% D and 72% D (17) for carboplatin and cisplatin, respectively. These amounts correspond with 0.22 and 0.24 μmol/m^2 of carboplatin and cisplatin, respectively. This observation is in agreement with tissue levels at 24 hours after administration of the MTD in nude mice as measured by Boven et al (10). This means that the amounts of both drugs reacted with plasma proteins and tumor and tissues are about the same at the MTD. Furthermore, the final half-life of total Pt, as measured by the least-squares method over the first 5 days, corresponded very well with the final half-life observed after short-term infusion of cisplatin ($t_{1/2}$, Days 1–5 = 5.3 days) (16). This suggests that similar Pt-protein reaction products were formed.

The resemblance of carboplatin and free Pt with respect to their half-lives, MRT, AUC, CL, and V_{ss} (table 2) indicates a slow metabolic conversion of carboplatin to species with a molecular weight < 30,000. Although the plasma concentrations of carboplatin and free Pt were almost equal, the urinary excretion (0–6 hours) of carboplatin was lower than that of free Pt. This may be caused by a lower glomerular filtration of carboplatin or by degradation of carboplatin in the bladder during the collection intervals of 2 hours (21).

The difference in carboplatin and free Pt concentrations from 9 hours after administration onwards provides evidence for the presence of low-molecular-weight metabolites, formed either by direct conversion of carboplatin or by degradation of protein-carboplatin complexes. The CL_M of free Pt (table 3) was in excellent agreement with the value of 23 ml/minute calculated by Calvert et al (3) in a totally different way.

K_M of free Pt appeared to be ten times higher for cisplatin than for carboplatin. Therefore, it can be concluded that in vivo cisplatin is about ten times more reactive than carboplatin. This factor of ten is in good agreement with the difference in reactivity towards the strong nucleophile sodium thiosulfate (36). Difference in reactivity was also thought to be the cause of a difference in binding to DNA (11) and cytotoxicity (12). In solutions a 100-fold-higher concentration of carboplatin was needed to reach equal levels of Pt-DNA intrastrand

crosslinks, which produced equivalent DNA damage after 24 hours of incubation (11). In cell suspensions, however, 20–40-fold more carboplatin than cisplatin was required to produce DNA binding and cytotoxicity to the same extent (11). This decrease in excess of dose may be explained by the fact that a relatively lower amount of cisplatin will reach DNA due to a difference in reactivity to cellular components on their way to the nucleus. Similarly, because even more alternative reactions of cisplatin will be possible, it can be understood that in vivo the excess of carboplatin needed to induce equal cytotoxicity is about tenfold as reflected by the observed ratio of the AUCs of free Pt in plasma after a dose of 350 and 100 mg/m^2 of carboplatin and cisplatin, respectively (16,17).

The concentration-time profile of Pt in rbcs also indicates a lower reactivity of carboplatin compared to cisplatin. Peak Pt concentrations in rbcs were lower than those observed after administration of 100 mg/m^2 of cisplatin [2.9 ± 0.4 μM (17)], despite the higher dose of carboplatin (350 mg/m^2). Peak levels were also reached at later time points.

It can be concluded that pharmacokinetics of carboplatin reflect its lower reactivity compared to cisplatin. By giving higher amounts of carboplatin to cancer patients a comparable extent of reaction with human body constituents may be achieved.

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Perturbation of In Vitro Drug Resistance in Human Lymphatic Neoplasms by Combinations of Putative Inhibitors of Protein Kinase C[1,2,3]

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Fresh specimens of human lymphatic neoplasms were tested with the differential staining cytotoxicity assay. Cells from relapsed patients with acute lymphoblastic leukemia (ALL) were significantly more resistant to vincristine, dexamethasone, and doxorubicin in the assay than were cells from previously untreated patients. The putative C kinase inhibitors verapamil (V), imipramine (I), lidocaine (L), tamoxifen (T), chlorpromazine (C), and haloperidol (H) were then tested singly, in combination with each other (VILTCH, ITCH, and VL), and in combination with vincristine. At concentrations judged to be clinically achievable, VILTCH itself was occasionally toxic to ALL and chronic lymphocytic leukemia. The VILTCH combination clearly potentiated the cytotoxic activity of vincristine in five of eight ALL specimens from relapsed patients and potentiated vincristine in 18 of 30 chronic lymphocytic leukemia specimens. It also potentiated vincristine in two of six specimens of multiple myeloma and five of six specimens of non-Hodgkin's lymphoma. The VILTCH combination had no significant effects in fresh cultures of normal human lymphocytes. The most active drugs in the VILTCH combination appeared to be verapamil and lidocaine. We conclude that the differential staining cytotoxicity assay is a useful tool to study the circumvention of clinically acquired drug resistance. While the mechanism of the observed enhancement of the cytotoxic effects of vincristine is not known, it is possible that combinations of putative C kinase inhibitors may reduce drug resistance in human lymphatic neoplasms. [Cancer Treat Rep 71:1239-1243, 1987]

It is now established that a number of nontoxic agents have the ability to decrease drug resistance in tissue culture systems. Most attention has centered on agents with the known capability of interacting with calcium channels or with calmodulin (1). Most of the agents are also known to have the capacity to interact with protein kinase C (C kinase) (2,3). These include drugs such as verapamil, phenothiazines, tamoxifen, local anesthetics, and other agents. These data and the work of other authors (4-7) suggest the possibility that C kinase may be involved with the regulation of drug resistance in these cells.

We previously described how the differential staining cytotoxicity (DiSC) assay accurately discriminates between drug-sensitive and drug-resistant cell populations in human lymphatic neoplasms (HLN) (8). We previously used the DiSC assay to study the ability of menadiol (vitamin K_3) to enhance the activity of standard antineoplastic agents in HLN (9). Accordingly, it was of interest to determine the effect inhibitors of C kinase would have on drug resistance in these same cells. Our results indicate that combinations of C kinase inhibitors have the capacity to reduce drug resistance to vincristine in acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and other types of HLN.

METHODS

Specimens, patients, and drugs.—Specimens included EDTA-anticoagulated blood, bone marrow specimens, or lymph node biopsies obtained in accordance with the

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Human Studies guidelines of the collaborating institutions. In most cases, these specimens represented aliquots of material removed for unrelated indications that were in excess of the quantities required for diagnostic purposes. When tissue was removed expressly for this study, prior Institutional Review Board approval and informed consent in accordance with local institution and National Institutes of Health guidelines were obtained. Cisplatin was obtained in powdered form from the Division of Cancer Treatment of the National Cancer Institute (Bethesda, MD). Dexamethasone and vincristine were prepared by diluting vials of clinical drug solutions. Verapamil, imipramine, lidocaine, tamoxifen, chlorpromazine, and haloperidol were obtained from commercial pharmaceutical sources. Drugs were prepared at ten to 100 times the desired final concentration and stored at −65°C until use.

Assay methodology.—Specimens were assayed for chemosensitivity with the DiSC assay as described elsewhere (8), which involves a 4-day continuous drug exposure in polypropylene tubes, staining dead cells with fast green/nigrosin, adding an internal standard of acetaldehyde-fixed duck rbcs, cytocentrifugation, counterstaining with Wright-Giemsa, and counting living neoplastic cells and duck rbcs. The assay end point is the total mass of viable tumor cells following drug exposure, relative to control cultures without drug. Modulating agents such as verapamil were added to the cell cultures 1 hour prior to the addition of the other drugs to be tested.

Modulating agents.—The following putative C kinase inhibitors were tested: verapamil hydrochloride (0.5 μg/ml), tamoxifen citrate (0.15), haloperidol lactate (0.1), chlorpromazine hydrochloride (0.75), lidocaine hydrochloride (6.0), and imipramine hydrochloride (0.5). Results were the same when drugs were added separately to the cultures or when they were first combined in solution and then added to the cultures. These concentrations are believed to be clinically achievable (10–22).

Definitions of drug interactions.—Owing to the complex design of our clinical assay protocols, we did not perform formal isobol analysis for drug synergy, addition, or antagonism. According to previous conventions (23), "synergy" or "potentiation" was said to occur when the effect of two treatments in combination exceeded the product of the individual effects of the two treatments tested separately. "Addition" ("additivity") was said to occur when the effect of two treatments in combination approximated the product of the individual effects of the two treatments tested separately.

RESULTS

We previously reported that specimens from relapsed ALL patients were more resistant to vincristine, dexamethasone, and doxorubicin in the DiSC assay than were specimens from previously untreated patients (8). Figure 1 shows updated results for vincristine. The mean percent cell survival for cells from untreated patients was 26% ± 4.5% SEM. The mean percent cell survival for cells from relapsed patients was 61.6% ± 4.8%. Corresponding median percents cell survival were 17% and 63%, respectively. Differences between corresponding means and medians were significant at $P < 0.001$ by t test and median test, respectively. Likewise, specimens from relapsed patients were significantly more resistant to dexamethasone and doxorubicin than were specimens from untreated patients (data not shown). We then proceeded to screen modulating agents for their ability to convert assay results from the "relapsed" range to results in the "untreated" range.

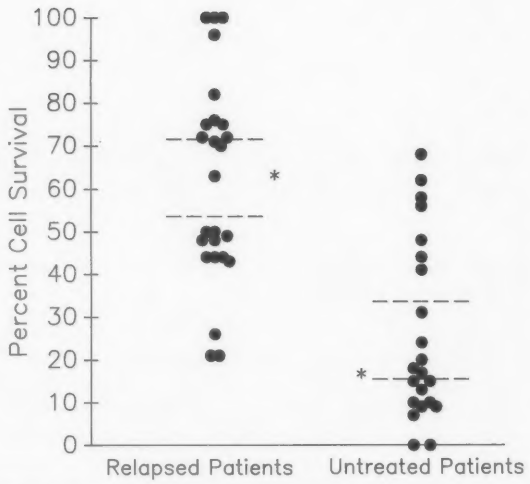

FIGURE 1.—% cell survival from assays performed on relapsed and untreated patients with ALL. Vincristine was tested at concentration of 0.08 μg/ml, with continuous exposure for 4 days. Each symbol represents different assay and different patient. Dashed lines represent 95% confidence levels of mean, and asterisks represent medians for relapsed and untreated assay groups. Both means and medians were significantly different, as described in text.

Verapamil (V), imipramine (I), lidocaine (L), tamoxifen (T), chlorpromazine (C), and haloperidol (H) were tested in combination with vincristine in ALL and CLL cells freshly obtained from patients. The drugs in VILTCH were combined to form a fixed combination at clinically achievable concentrations (*see* Methods section). In this form, the VILTCH combination was toxic (< 30% cell survival) to two of four specimens from untreated ALL patients (fig 2); to none of eight specimens from relapsed ALL patients (fig 3); to none of ten specimens of normal lymphocytes (table 1); to six of 31 CLL specimens (table 2); to none of six multiple myeloma (MM) specimens (table 3); and to one of six non-Hodgkin's lymphoma (NHL) specimens (table 3). The VILTCH combination either had no effect or was only additive with vincristine in all four specimens from untreated ALL patients (fig 2), but it clearly potentiated vincristine in five of eight specimens from relapsed ALL patients (Nos. 6, 7, 9, 10, and 12) (fig 3). It poten-

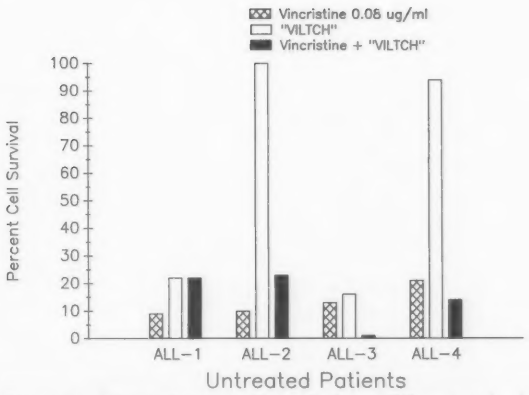

FIGURE 2.—Results of assays on blast cells from 4 different previously untreated ALL patients.

tiated vincristine in 18 of 30 CLL specimens (table 2), in two of six MM specimens (table 3), and in five of six NHL specimens (table 3) but did not potentiate vincristine in normal lymphocytes (table 1).

To determine the active moiety in the VILTCH combination, the following combinations were tested in CLL cells: VILTCH, ITCH, VL, verapamil alone, and lidocaine alone. The data in figure 4 suggest that verapamil and lidocaine were the most important agents in the VILTCH combination.

DISCUSSION

Untreated ALL patients are often curable with standard chemotherapy based on vincristine, prednisone, and anthracyclines. In contrast, relapsed patients are virtually incurable with similar chemotherapy. It is, therefore, obvious that leukemia cells in relapsed patients are, as a class, more drug resistant than are leukemia cells in previously untreated patients, as a class. The DiSC assay correctly identified specimens from relapsed patients as being significantly more drug resistant to vincristine, dexamethasone, and doxorubicin

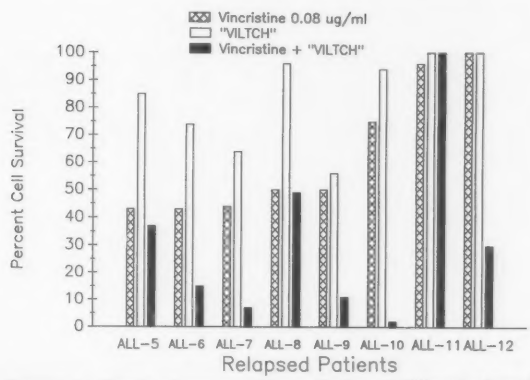

FIGURE 3.—Results of assays on blast cells from 8 different relapsed ALL patients.

TABLE 1.—Effects of Vcr, VILTCH, and Vcr + VILTCH on fresh specimens of normal lymphocytes*

Assay specimen	% cell survival		
	Vcr (0.08 μg/ml)	VILTCH	Vcr + VILTCH
1	54	92	69
2	87	74	60
3	97	93	83
4	103	104	106
5	114	100	70
6	63	122	64
7	70	85	67
8	101	74	100
9	79	88	78
10	139	93	118
Mean	91	93	82
SD	26	14	20
SEM	9	5	7

*Vcr = vincristine.

TABLE 2.—Effects of Vcr, VILTCH, and Vcr + VILTCH on fresh specimens of CLL

Assay specimen	% cell survival		
	Vcr (0.08 μg/ml)	VILTCH	Vcr + VILTCH
1	64	18	3
2	5	84	4
3	14	9	5
4	86	15	5
5	81	70	5
6	78	66	9
7	89	87	9
8	89	27	11
9	33	28	11
10	31	87	11
11	42	57	12
12	63	56	14
13	63	56	14
14	45	37	17
15	38	44	18
16	51	52	20
17	37	62	20
18	29	19	22
19	91	86	23
20	60	92	28
21	88	50	36
22	75	87	39
23	126	123	40
24	60	74	44
25	69	93	47
26	96	90	62
27	70	116	87
28	88	106	99
29	43	85	104
30	159	122	131
Mean	65	67	32
SD	32	32	32
SEM	5.4	6	6.2

TABLE 3.—Effects of Vcr, VILTCH, and Vcr + VILTCH on fresh specimens of MM and NHL

Patient No.	Neoplasm	% cell survival		
		Vcr (0.08 μg/ml)	VILTCH	Vcr + VILTCH
1	MM	46	144	8
2	MM	37	89	9
3	MM	35	107	22
4	MM	27	113	11
5	MM	19	96	19
6	MM	5	86	8
1	NHL	66	20	3
2	NHL	58	73	23
3	NHL	36	59	5
4	NHL	36	86	8
5	NHL	28	78	6
6	NHL	5	77	1

than were specimens from previously untreated patients. These data indicate that the DiSC assay can be used as a model for drug resistance in human neoplasms, as drug resistance is acquired in the course of clinical chemotherapy. Established cell line models of drug resistance have not clearly modeled clinical drug resistance (24). We hypothesized that the DiSC assay in HLN might more successfully identify strategies for circumventing drug resistance.

We screened six putative protein kinase C inhibitors in combination (VILTCH) for the ability to potentiate vincristine in relapsed specimens of ALL and in other HLN. When tested at concentrations projected to be clinically achievable (10–22), the VILTCH combination was highly active in reducing resistance to vincristine in ALL. The VILTCH combination converted the mean percents cell survival of relapsed ALL specimens from the range characteristic of relapsed patients to the range characteristic of previously untreated patients (figs 1–3). In this respect, VILTCH can be said to have "reversed" acquired drug resistance in fresh human ALL specimens. Similar potentiation of vincristine was frequently observed in specimens of other types of HLN as well.

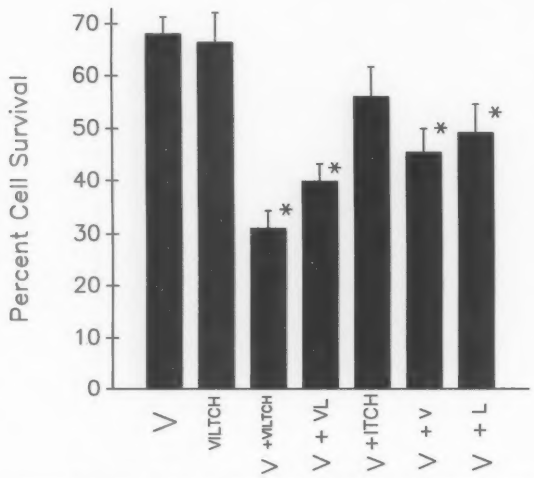

FIGURE 4.—Mean ± SEM of assays on CLL cells. Drugs tested were vincristine (V) alone (0.08 μg/ml, 66 specimens); VILTCH alone (concentrations listed in Methods section, 31 specimens); V + VILTCH (56 specimens); V + verapamil + lidocaine (V + VL, 22 specimens); V + ITCH (19 specimens); V + V (16 specimens); and V + L (16 specimens). Values represented by bars with asterisks are significantly less than value for bar representing V alone; at least $P < 0.05$ by t test.

The most active drugs in the VILTCH combination appeared to be verapamil and lidocaine, as studied in CLL (fig 4), and the simplified combination of verapamil and lidocaine was modestly less effective than VILTCH in CLL. The combination of verapamil and lidocaine is appealing from a clinical point of view, as these agents have different effects on electrical conduction and myocardial contractility and probably do not have overlapping toxic effects. Even if these two agents are less than additive in combination, the use of the drugs in combination would increase the probability that effective clinical concentrations of at least one agent would be maintained during cancer chemotherapy. The VILTCH combination was, by itself, infrequently toxic in assays performed in HLN. Other authors have previously observed that phenothiazines (25–27) and lidocaine (28) may have cytotoxic effects in other types of tumor systems. The individual phenothiazine and local anesthetic agents have not, at least by themselves, been confirmed to have activity in clinical human cancer.

In these initial studies, we have obviously tested highly complex combinations of agents. It would be difficult to sort out precise mechanisms for the enhanced cytotoxicity observed in our studies. Although the agents were selected because of the speculation that protein kinase C inhibitors might perturb drug resistance, it is by no means certain that the mechanism of action will prove to have association with C kinase. For example, there is growing evidence that sodium transport may be related to acquired drug resistance in established cell lines (29), and this would offer an alternative mechanism of action for agents such as lidocaine.

In conclusion, we feel that our data indicate (*a*) that the DiSC assay may be used as a tool for the study of clinically acquired drug resistance in HLN and (*b*) that resistance modification based on the use of combinations of putative C kinase inhibitors may be a worthwhile approach to the problem of circumventing acquired drug resistance in HLN. Clinical trials of the verapamil-lidocaine combination are currently ongoing at the institutions affiliated with the University of California Irvine.

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Phase I Trial of Homoharringtonine in Children With Refractory Leukemia[1]

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Continuous infusion of homoharringtonine was administered to 17 children with refractory leukemia. Ten children with acute lymphoblastic leukemia received a total of 18 courses and seven children with acute nonlymphoblastic leukemia had a total of 13 courses. Doses were escalated from 1.65 to 8.5 mg/m^2 for 5–10 consecutive days. Side effects included mild nausea and vomiting and transient changes in liver enzymes. Mucositis and diarrhea were more frequently seen at higher dose levels. Grade 3 hypotension and pain were seen at doses of 7 mg/m^2 for 10 days. This is considered to be the maximum tolerated dose in this limited phase I trial. None of these previously heavily treated patients achieved a marrow remission. [Cancer Treat Rep 71:1245–1248, 1987]

Homoharringtonine (HHT) is one of several alkaloids isolated from the evergreen tree *Cephalotaxus fortunei* Hook f., which is indigenous to China (1–5). HHT has shown antitumor activity against murine tumors, P388 leukemia, and colon 38 tumor cells (6,7). There was moderate activity against L1210 leukemia and $CD8F_1$ mammary carcinoma and slight activity against B16 melanoma. Its mechanism of action may be through inhibition of protein and nucleic acid synthesis at the level of chain initiation (8). Preclinical pharmacology in dogs and mice has indicated that the major toxic effects were hematologic and cardiac (9). In clinical trials in China, remissions in leukemia were reported in both newly diagnosed and relapsed patients (10–12). The relatively low toxicity and reported high rates of remission induction prompted the undertaking of phase I and II trials of this agent in the United States. A wide range of dose schedules have been used in the treatment of leukemia (13) and solid tumor (14–17) patients. Doses ranged from 0.2 to 9 mg/m^2/day given as single infusions every 21 days to 5 or 10 days of continuous infusion of the agent. Since rapid infusions of HHT can produce serious hypotension (14) and cardiac arrhythmias, we evaluated the continuous infusion of HHT in 17 children with advanced leukemia.

PATIENTS AND METHODS

Patients who relapsed on chemotherapy and who have been off all previous therapy for 4 weeks and/or have recovered from its toxicity were eligible for study after informed consent was obtained. Adequate renal, hepatic, and cardiac functions (serum creatinine $\leq$ 1.5 mg/dl, bilirubin $\leq$ 3 mg/L, and normal echocardiogram and ECG) and a projected life expectancy of 8 weeks were required. All patients had a complete history and physical examination.

Follow-up evaluation included a detailed daily physical examination with cbc, platelet count, and differential. A 12-channel biochemical screening, 5′-nucleotidase, glucose, γ-GPT, electrolytes, and creatinine data were obtained twice weekly. Bone marrow aspiration and biopsy were performed on Days 14 and 21. Patients had echocardiogram and ECG before every course of treatment and more frequently when indicated by the clinical course. All patients were hospitalized and placed on a cardiac monitor during treatment. Vital signs were measured every 4 hours during the drug infusion. The prescribed daily dose of HHT was diluted into 500–1000 ml of 5% dextrose solution. The drug was infused into venous catheters (if present) or peripheral veins over 24 hours using an infusion pump.

Patient characteristics are shown in table 1. The number of previous bone marrow relapses ranged from one to five (median, three relapses).

Criteria for therapeutic response were based on a study by Hewlett et al (18). Toxicity was graded according to the modified criteria for pediatric patients by Miller et al (19). The toxicity grading used for the side effects observed in this study is summarized in table 2.

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total entered	17
Median age in yrs (range): 9 (1–17)	
Sex	
Male	12
Female	5
Diagnosis*	
ALL	10
ANLL	6
Chronic myelocytic leukemia → blastic	1

*ALL = acute lymphoblastic leukemia; ANLL = acute nonlymphoblastic leukemia.

RESULTS

Table 3 shows the dose levels in mg/m^2 for 5–10 days. The initial dose of HHT was 1.65 mg/m^2/day for 5 consecutive days. The next dose level was 4.5 mg/m^2/day for 5 days. At these dose schedules there was only a brief decrease in peripheral wbc count. Because of this very transient "oncolytic effect," the dose of HHT was escalated to 6.0, 7.0, and 8.5 mg/m^2 and the duration of administration was increased first to 7 and then to 10 consecutive days. Treatment was repeated every 14–21 days if the patient recovered from toxicity. Dose escalation within the same patient was permitted. Thus, ten children with ALL and seven with ANLL received a total of 31 courses.

Nonhematologic Toxic Effects

The major side effects of HHT observed in this study were gastrointestinal (nausea and vomiting, mucositis, diarrhea, and hepatic toxicity), skin lesions, hypotension, and pain.

Nausea and vomiting was mostly mild (grade 2), except in one child who had grade 3 at the highest dose level. Mild mucositis was seen on Days 4–20 of four courses at total dose levels $\geq$ 45 mg/m^2 given in 10 days. Diarrhea, which occurred toward the end of the treatment course, also appeared to be dose-related. Hepatic toxicity included dose-related transient elevation of SGOT and/or bilirubin.

Skin lesions were seen in patients who received doses $\geq$ 60 mg/m^2 given in 10 days. Skin erythema over the abdomen, groin, and extremities developed on Days 2–13 (median, Day 6) of the HHT infusion. This was followed by generalized maculopapular rash which lasted for 7–10 days, then peeling.

Hypotension of grade 1 or 2 occurred in most of the patients beginning the second or third day of HHT infusion. Blood pressure was usually returned toward baseline level either by interrupting the HHT or decreasing the infusion rate. No volume expansion or vasopressors were necessary.

At the initial dose level, only one child was entered who had no side effects after the first course, and she received a second course 2 weeks later. On Days 3 and 4 of the second course, blood pressure decreased from 100/70 to 88/54 mm Hg, but remained asymptomatic at 90/60 mm Hg thereafter.

Two children had grade 3 hypotension at a dose level of 70 mg/m^2 given in 10 days. One was a patient with ALL in his third bone marrow relapse. On Day 3 of HHT infusion, his blood pressure decreased from a baseline of 100/50 to 80/50 mm Hg. He became edematous and complained of chest pain. HHT was then interrupted. After receiving albumin, his symptoms disappeared. HHT was resumed and he completed the 10-day course. He also developed generalized pain which required morphine for control. On Day 2 of HHT infusion, the other child, with ALL in his second bone marrow relapse, developed hypotension associated with tachycardia and dizziness. With the decrease in HHT infusion rate he completed the treatment course. His other side effects included mucositis, diarrhea, skin lesions, and generalized pain.

TABLE 2.—Toxicity grading

Toxic effect	Grade 1	Grade 2	Grade 3	Grade 4
Nausea and vomiting	Nausea	Transient vomiting	Vomiting, requiring hydration	Intractable vomiting
Mucositis	Soreness, erythema	Erythema, ulcers, can eat solid foods	Ulcers, liquid diet only	Alimentation not possible
Diarrhea	< 2 days, no therapy	Tolerable, > 2 days	Intolerable, requiring therapy	Hemorrhagic, dehydration
Skin	Erythema	Dry desquamation, vesiculation	Moist desquamation, ulceration	Exfoliative dermatitis, necrosis
Hepatic: bilirubin/SGOT/SGPT	1.25–2.5 × normal	2.6–5 × normal	5.1–10 × normal	> 10 × normal
Hypotension	< 20% decrease from baseline	> 20% decrease from baseline	Symptomatic, recovers after decreased/discontinued therapy	Unresponsive to intervention
Pain	Mild	Moderate	Severe	Excruciating

TABLE 3.—Toxic effects

Dose level		No. of—		Toxic effect*						
mg/m² × days	(total)	Patients entered	Evaluable courses	Nausea and vomiting	Mucositis	Diarrhea	Hepatic	Skin lesions	Hypotension	Pain
1.65 × 5	(8.25)	1	2						1 (2)	
4.5 × 5	(22.5)	3	5	3 (2)		1 (1)	1 (1)		3 (1), 1 (2)	1 (3)
4.5 × 7	(31.5)	3	4	2 (2)			1 (1)		3 (1)	
4.5 × 10	(45.0)	3	4	2 (1), 1 (2)	1 (1)		2 (1)		2 (1,2)	
6.0 × 10	(60.0)	3	4	2 (1,2)	1 (2)		1 (2), 2 (3)	2 (2)	2 (1)	
7.0 × 10	(70.0)	5	8	5 (2)	1 (1)	3 (2), 1 (3)	3 (2,3,4)	4 (2)	2 (1), 2 (2), 2 (3)	3 (2,3,4)
8.5 × 10	(85.0)	4	5	1 (3)	1 (2)	2 (2)	2 (1), 1 (3)	3 (1,2,3)	1 (1)	1 (3)

*Values = No. of courses with toxicity (grade).

Generalized myalgic pain occurred on Day 1, 3, or 4 of HHT infusion and lasted for 3–12 days. All patients required pain medication. Pain was seen in four courses of the nine patients who received ≥ 70 mg/m² given in 10 days. The one child who had grade 3 pain received HHT at 22.5 mg/m² in 5 days as his second course. This patient had grade 4 pain during his first course of HHT at 70 mg/m² in 10 days.

Cardiac complication was observed in an 11½-year-old boy with ALL in second bone marrow relapse. His prior total dose of doxorubicin was 540 mg/m² and daunorubicin was 60 mg/m². A course of HHT at 70 mg/m² given in 10 days was well tolerated except for a grade 2 skin rash. There was no change in peripheral blood cell counts or bone marrow. He required multiple transfusions. Three weeks later he was given a second course of HHT at 85 mg/m² in 10 days. On Day 10 of the continuous infusion of HHT he developed transient, precipitous hypotension of 70/36 mm Hg concomitant with epistaxis and hematemesis. Two days later, after stabilization, ECG showed atrial ventricular block varying in severity from prolonged PR interval, Wenckebach's disease, and complete heart block associated with occasional premature ventricular contractions. There was a decrease in peripheral wbc count but no change in bone marrow. He died of progressive disease 1 month later. Permission for autopsy was not obtained. Cardiac biopsy demonstrated some cytoplasmic vacuolization of the myocytes which were the type seen with anthracyclines. The electron microscopy of the heart shows vacuolization of the sarcoplasma and electron-dense paranuclear inclusions. The nature of the latter is not known.

Hematologic Toxic Effects

There were ten children with ALL who received a total of 17 courses of HHT at 4.5 mg/m²/day × 5 to 8.5 mg/m²/day × 10 days. In eight of the courses there was a decrease of peripheral wbc count but no change in blast count of the bone marrow. In the other nine courses, the wbc count was low prior to HHT: in two courses patients had a rapid increase in peripheral blasts, and in seven courses patients had little or no change in marrow. This included a patient whose marrow was hypocellular by biopsy but had 95% blasts. On Day 17, after a course of HHT at 70 mg/m² given in 10 days, his marrow showed 9.5% blasts, which lasted for 2 weeks only. There were seven children with ANLL who received a total of 13 courses from 1.65 mg/m²/day × 5 to 8.5 mg/m²/day × 10. In eight of the courses there was a transient decrease in peripheral wbc count but no change in the bone marrow. In the other five courses, wbc count was low prior to HHT and their bone marrow became hypocellular after HHT but had blasts.

CONCLUSION

HHT was given by continuous infusion over 5–10 days to 17 children with refractory leukemia. Gastrointestinal toxicity was usually mild; nausea and vomiting, mucositis, diarrhea, and transient elevation of bilirubin or liver enzymes occurred during the treatment course. Skin rash was seen in patients receiving higher dosages and disappeared within 2 weeks. Hypotension has been seen in adult and in pediatric patients. In this study, grade 1 or 2 hypotension was seen at all dose levels, but two patients were symptomatic at 70 mg/m² given in 10 days. In six of the 31 treatment courses, HHT infusion had to be interrupted. The dose-limiting toxic effect was pain. Most patients required continuous morphine for pain relief during the 10 treatment days. Since hypotension and pain occurred at doses ≥ 70 mg/m² given in 10 days and these symptoms debilitate the patients, we consider this the maximally tolerated dose.

Although in some patients there was decrease in peripheral wbc count, only one child with ALL had a brief M_2 marrow. None of these patients achieved marrow remission. It is possible that these pediatric patients were more heavily pretreated than those reported in a concurrent adult trial at this center (13).

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Overview of Current Status and Future Direction of Clinical Trials With 5-Fluorouracil in Combination With Folinic Acid[1]

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The drug 5-fluorouracil (5-FU) has antineoplastic activity against a broad spectrum of solid tumors and is widely used in the clinic as a single agent as well as in combination with other agents in the treatment of gastrointestinal malignancies, breast cancer, and squamous cell carcinoma of the head and neck. Administered as a single agent, 5-FU has been the standard therapy for patients with metastatic colorectal carcinoma. Unfortunately, the efficacy of 5-FU is limited, with an objective response rate in the range of only 10%–25%.

The elucidation of the complex intracellular metabolism of 5-FU has provided insights into biochemical mechanisms of cellular resistance to this antimetabolite that suggest strategies to circumvent resistance through pharmacologic modulation. The combination of 5-FU with folinic acid (leucovorin, citrovorum factor) represents one such strategy. The preclinical rationale and the clinical experience with 5-FU and folinic acid are reviewed, and the unresolved issues pertaining to the clinical use of this combination are discussed.

PRECLINICAL RATIONALE

The drug 5-FU exerts its cytotoxic effects by at least two mechanisms of action: incorporation of 5-FU as FUTP into RNA and inhibition of thymidylate synthetase (TS) (fig 1) (1–3). In addition, it has been shown that 5-FU can be incorporated into DNA, and it is possible that this effect may also contribute to cytotoxicity (4–6). The inhibition of TS by the 5-FU metabolite FdUMP results in inhibition of the de novo production of dTMP from dUMP; depletion of dTTP pools consequently interferes with DNA synthesis. The ability of FdUMP to inhibit TS can be affected by several parameters, including the amount of FdUMP and the level of the substrates dUMP and 5,10-methylenetetrahydrofolate (5,10-CH_2-FH_4).

Reduced folate coenzymes are involved in one-carbon transfer reactions such as those necessary for the biosynthesis of methionine, serine, dTMP, and purines (7). The 5,10-CH_2-FH_4 cofactor is necessary for potent inhibition of TS by FdUMP through the formation of a covalent ternary complex. The intracellular pool of 5,10-CH_2-FH_4 is derived from metabolic conversion of circulating reduced folates that can enter the cell via a carrier-mediated transport mechanism (fig 2). The concentration of the normal circulating plasma folate 5-methyltetrahydrofolate (5-CH_3-FH_4) is 0.01–0.02 μM, and the intracellular concentration of 5,10-CH_2-FH_4 has been estimated to be in the range of 0.066–0.233 μM (7,8). The interconversions of the folate cofactor pools in vivo are complex, and little is known about the overall mechanism of cellular folate homeostasis.

The failure either to form or to maintain a stable ternary complex is a potential mechanism of resistance to 5-FU. The cytotoxicity of 5-FU and FdUrd in several preclinical models has been enhanced by coadministration of exogenous reduced folate (8–13). For example, Houghton et al (9) characterized several human colorectal adenocarcinoma xenografts with respect to their sensitivity to 5-FU and correlated this with the formation of covalent ternary complex. In nonresponsive tumors, maximal ternary complex formation resulting in complete inhibition of TS could only be achieved by the addition of exogenous reduced folate. In responsive tumors, in contrast, maximal binding of FdUMP into the ternary complex occurred without exogenous cofactor.

Ullman et al (10) reported that the addition of 1 μM folinic acid to FdUrd increased the cytotoxicity fivefold in murine L1210 leukemia cells. In addition, the ternary complex formed was quantitated in L1210 cells continuously grown in folate-free medium. The cells were suspended in folate- and serum-free medium containing 0.5 μM [6-^{3}H]FdUrd ± 20 μM folinic acid, and the amount of ternary complex formed after a 40-minute incubation was determined by sodium dodecylsulfate electrophoresis. Cells exposed to exogenous folinic acid had a 12-fold increase in the amount of covalent complex, compared to that in cells treated without folinic acid (10).

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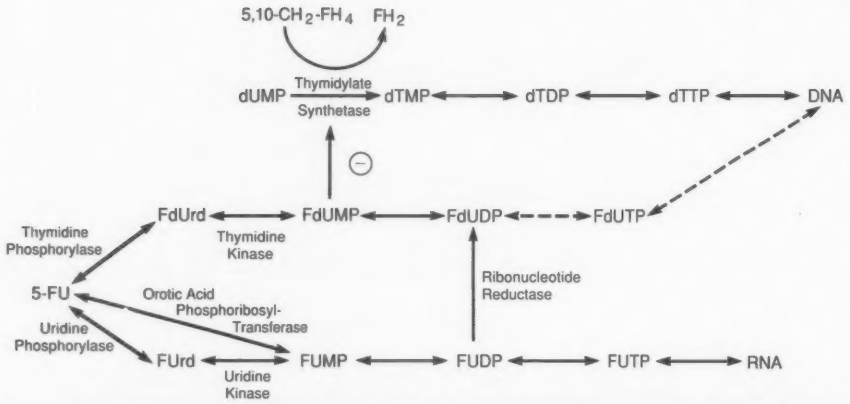

FIGURE 1.—Metabolism of 5-FU. dUMP = deoxyuridine monophosphate; dTMP = thymidylate; dTDP = thymidine diphosphate; dTTP = thymidine triphosphate; FdUrd = fluorodeoxyuridine; FdUMP = fluorodeoxyuridine monophosphate; FdUDP = fluorodeoxyuridine diphosphate; FdUTP = fluorodeoxyuridine triphosphate; FUrd = fluorouridine; FUMP = fluorouridine monophosphate; FUDP = fluorouridine diphosphate; and FUTP = fluorouridine triphosphate.

Evans et al (11,12) compared the effect of exogenous reduced folates on the cytotoxicity of 5-FU in mouse sarcoma (S-180) and human carcinoma cells (Hep-2) in vitro. The S-180 cells are 50 times more sensitive to 5-FU than Hep-2 cells following a 3-hour exposure to 5-FU: the concentrations of 5-FU associated with 50% inhibition of cell growth, compared to controls, were 4.7 and 228 μM, respectively. The TS inhibition is thought to be growth limiting in S-180 cells since the S-180 cells can be rescued by exogenous thymidine (11). The cytotoxicity of 5-FU (30 μM for 3 hrs) could be increased threefold in both cell lines by simultaneous exposure to pharmacologic concentrations of reduced folates (12). The lowest concentration of folinic acid that provided for maximum potentiation was 10 μM; 300 μM folic acid and 10 μM $5\text{-}CH_3\text{-}FH_4$ produced effects similar to those of folinic acid. The potentiating effect of folinic acid on growth inhibition by 5-FU appeared to result from slower recovery of dTMP synthesis following removal of 5-FU.

In these preclinical studies, the interaction of 5-FU with $5,10\text{-}CH_2\text{-}FH_4$ cofactor in normal tissues has not been carefully examined to determine whether the interaction is likely to increase the selectivity of the combination. Furthermore, the optimal duration of exposure to folinic acid for maximum inhibition of TS by FdUMP has not been precisely defined by preclinical models. The rate of dissociation of the ternary complex is inversely proportional to the concentration of reduced folate in equilibrium with the complex (14). Yin et al

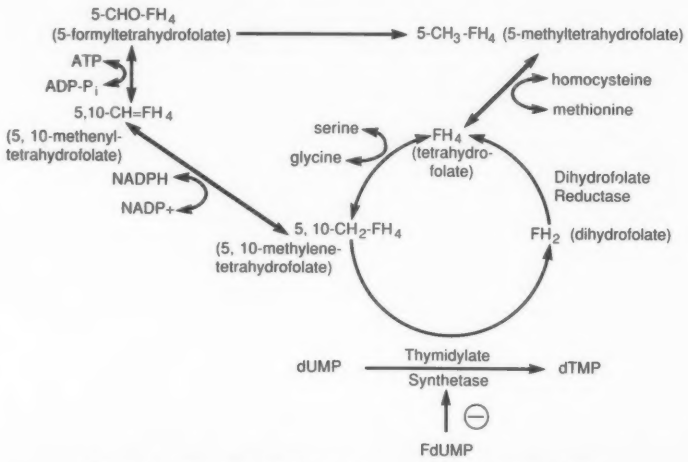

FIGURE 2.—Simplified schema for interconversions of reduced folates.

(15) have demonstrated that intracellular levels of reduced folates accumulate slowly when cells are continuously exposed to folinic acid in tissue culture. Furthermore, it has been shown that mammalian cells that have been preloaded with labeled folylmonoglutamates rapidly lose the reduced folates into the medium by an efflux mechanism (16). Such observations suggest that a prolonged continuous infusion (CI) of folinic acid theoretically might be needed to maintain elevated levels of reduced folate cofactors. If the reduced folates are quickly metabolized to the polyglutamate form, however, the folylpolyglutamates can accumulate intracellularly above the levels of the parent molecule that would otherwise exist in equilibrium with the extracellular reduced folate (17). Under such circumstances, bolus administration or short-term infusion of a high dose of folinic acid would be sufficient to increase the total reduced folate pool intracellularly.

PHARMACOKINETICS AND CELLULAR PHARMACOLOGY OF FOLINIC ACID

Prior to discussing the clinical trials of 5-FU and folinic acid, it would be useful to review the biochemical pharmacology of reduced folates. Folic acid is a vitamin that, following absorption from the gastrointestinal tract, must be enzymatically reduced through dihydrofolate (FH_2) to tetrahydrofolate (FH_4), an active coenzyme form that can act as an acceptor of one-carbon units. Folinic acid is a chemically synthesized form of reduced folate that consists of equal amounts of the diastereoisomers *d*- and *l*-5-CHO-FH_4. The natural diastereoisomer is *l*-5-CHO-FH_4. Folinic acid must be metabolized to exert its modulatory effects on methotrexate and fluoropyrimidine toxicity (fig 2). Straw et al (18) described the pharmacokinetics of *d,l*-5-CHO-FH_4 following iv administration of 25–100 mg in normal subjects. Folinic acid and its metabolites can be separated and quantitated by high-performance liquid chromatography (HPLC) methods, but microbial assays are necessary to distinguish between the diastereoisomers. The *l*-5-CHO-FH_4 was rapidly cleared from plasma by conversion to its metabolite 5-CH_3-FH_4 and by urinary excretion (table 1). In contrast, *d*-5-CHO-FH_4 did not appear to be significantly metabolized, and its plasma half-life greatly exceeded that of the *l*-isomer and its metabolite *l*-5-CH_3-FH_4. One hour following an iv dose of 50 mg of folinic acid, the plasma concentrations of *l*-5-CHO-FH_4 and 5-CH_3-FH_4 were 2.1 and 1 μM, respectively.

Both diastereoisomers of folinic acid and 5-CH_3-FH_4 can be transported across the cell membrane by a common reduced folate carrier, although the results of competitive inhibition experiments indicate that *d*-5-CHO-FH_4 competes much less effectively with the natural diastereoisomer for transport in several murine tumor cell lines in vitro (19). The natural reduced folates subsequently undergo complex intracellular metabolism. A process termed polyglutamation, the addition of glutamate chains to the inherent molecular structure, may ensue. Polyglutamation of the reduced folate cofactors traps the molecules within cells, whereas the monoglutamate forms can efflux from the cell. The *l*-5-CHO-FH_4 can be metabolized to 5,10-CH_2-FH_4, the natural cofactor for the reaction involving conversion of dUMP into dTMP by TS. The 5-CH_3-FH_4 can also be converted to 5,10-CH_2-FH_4. It has been assumed that *d*-5-CHO-FH_4 is devoid of biologic activity. Both *d*- and *l*-5-CHO-FH_4, however, have been shown to be active substrates for the intracellular enzyme folylpolyglutamate synthetase (20). It has not been clearly determined whether the 5-CHO-FH_4 or 5-CH_3-FH_4 molecules can directly interact with TS in vivo without further metabolism, although Houghton et al (21) reported that 5-CHO-FH_4 and 5-CH_3-FH_4 were not effective in stabilizing the ternary complex in situ in cytosols derived from human colon carcinoma xenografts. The results from an in vitro model indicated that 10 μM *l*-5-CHO-FH_4 was as effective as 20 μM *d,l*-5-CHO-FH_4 in causing stabilization of the TS-FdUMP complex, and the investigators concluded that *d*-5-CHO-FH_4 was not responsible for the potentiation of fluoropyrimidine toxicity (22).

The composition of intracellular folate pools derived from radiolabeled folinic acid in vitro has been determined. Allegra et al (23) reported that 5-CH_3-FH_4 and FH_4 accounted for 47% and 18%, respectively, of the reduced folate pool in MCF-7 human breast cancer cells following a 24-hour exposure to 0.01 μM [^{3}H]5-*d,l*-CHO-FH_4. The plasma level of reduced folates in vivo, however, provides only an indirect reflection of intracellular folate pools (23).

INITIAL CLINICAL STUDIES

A number of investigators have attempted to translate the preclinical observation that an excess of intracellular reduced folates may be necessary for optimal inhibition of TS into the clinical setting in an effort to enhance the activity of 5-FU in colorectal carcinoma. Several different schedules of 5-FU in combination with folinic acid have been explored in the initial phase I/II studies (table 2) (24–39).

Bruckner et al (24–26) have conducted a series of phase I/II studies of 5-FU given in combination with iv folinic acid. A 10-minute infusion of 25 mg of folinic acid given 1 hour prior to 5-FU (925–1300 mg/m^2) was found to have acceptable toxicity, predominantly mild stomatitis and mild-to-moderate leukopenia (24). The combined complete response (CR) and partial response (PR) rate in 19 patients with previously untreated colorectal cancer was 16% (one CR and two PRs), but no PRs were seen in 16 previously treated patients (24). A subsequent study was conducted in which doses of folinic acid and 5-FU were independently altered. The dose of folinic acid was 25, 100, or 200 mg, accompanied by doses of 5-FU of 1300, 1110, or 925 mg/m^2, respectively. The overall response rate (RR) in 32 previously untreated patients was 22% (26). Pharmacokinetic studies using HPLC methodology indicated that following a 10-minute

TABLE 1.—Pharmacokinetic data on iv and oral folinic acid*

Folinic acid dosage	*d,l*-5-Formyl-FH_4	*d*-5-Formyl-FH_4	*l*-5-Formyl-FH_4	*l*-5-Methyl-FH_4
		Straw et al (18)		
50 mg iv over 4.5 mins				
Plasma level at 60 mins	NA	3 μg/ml (~ 6.2 μM)	1 μg/ml (~ 2.1 μM)	0.4–0.5 μg/ml (~ 0.8–1.0 μM)
V_d		7.9 ± 0.4 L†	17.5 ± 1.1 L†	22.9 ± 2.7 L†
$t_{½}$		451 ± 24 mins†	31.6 ± 1.1 mins†	227 ± 20 mins†
Cl_p		12.7 ± 1 ml/min†	390 ± 14 ml/min†	77.5 ± 9 ml/min†
Cl_r		12.7 ± 1.4 ml/min†	86 ± 6.4 ml/min†	32.2 ± 2.9 ml/min†
AUC		2303 mg/L/min	78.3 ± 7.4 mg/L/min†	310 ± 35 mg/L/min†
25 mg orally every 8 hrs × 9				
Peak plasma level	NA	500 ng/ml (~ 1 μM)		700 ng/ml (~ 1.4 μM)
$t_{½}$		481 ± 51 mins†		310 ± 25 mins†
AUC		193 ± 26 mg/L/min†	0.89 ± 0.17 mg/L/min†	226 ± 14 mg/L/min†
		Bruckner et al (26)		
100 mg over 10 mins				
Plasma concentration		NA	NA	NA
30 mins	14.0 μM			
60 mins	4.5 μM			
120 mins	2.5 μM			
		Trave et al (40)		
500 mg/m^2 over 120 mins				
Peak plasma concentration	NA	97.7 ± 34.3 μM†	28.8 ± 12.7 μM†	20.8 ± 6.8 μM†
$t_{½}$		534 mins	96 mins	
		Machover et al (29)		
200 mg/m^2/day × 5 by iv bolus				
Peak plasma concentration	NA	80–90 μM	43 μM	1–2 μM
$t_{½}$		659 ± 59 mins†		
$t_{½}a$			20 ± 2 mins†	
$t_{½}b$			122 ± 20 mins†	362 ± 59 mins†
		Newman et al (42)		
500 mg/m^2/day × 6 by CI	NA			
Cp_{ss}		40 ± 5 μM†	4.5 ± 0.5 μM†	4.7 ± 0.9 μM†
$t_{½}$		522 ± 167 mins†	53 ± 16 mins†	167 ± 67 mins†
		Herrera-Ornelas et al (33)		
500 mg/m^2/day × 5 by CI				
Cp_{ss}	30.0 μM	29.4 μM	0.6 μM	NA

*V_d = volume of distribution; $t_{½}$ = plasma half-life; Cl_p = plasma clearance; Cl_r = renal clearance; AUC = area under plasma concentration-time curve; NA = not available; and Cp_{ss} = steady-state plasma concentration(s).
†Values = mean ± SD.

infusion of 100 mg of folinic acid, the plasma levels of *d,l*-5-CHO-FH_4 decreased rapidly (table 1) (26).

Madajewicz et al (27) at Roswell Park Memorial Institute (RPMI) employed high-dose folinic acid (500 mg/m^2) given as a 2-hour infusion in combination with escalating doses of 5-FU administered as a bolus halfway into the infusion. This high-dose regimen was selected to reproduce the in vitro conditions suggesting that a minimum extracellular concentration of 10 μM 5-CHO-FH_4 and/or 5-CH_3-FH_4 for ≥ 3 hours was necessary for potentiation of 5-FU cytotoxicity. The courses were repeated weekly for 6 weeks followed by a 2-week rest. The maximum tolerated dose of 5-FU was 750 mg/m^2, and the recommended phase II dose was 600 mg/m^2. The dose-limiting toxic effects were leukopenia and gastrointestinal toxicity, mainly diarrhea. Other side effects included mild conjunctivitis and lacrimation, thinning of the nails, and alopecia. Among 23 evaluable patients with colorectal cancer, nine (39%) had PR; six of these patients had had prior 5-FU therapy. The median duration of response was 10 months (range, 6–18). Pharmacologic data derived from both HPLC and microbiologic assays indicated that a concentration of *l*-5-CHO-FH_4 > 10 μM was maintained for approximately 2 hours, suggesting that adequate levels of reduced folates required in vitro for potentiation of TS inhibition could be achieved in plasma (table 1) (40).

Another trial utilizing the weekly regimen was conducted at the Cleveland Metropolitan General Hospital in collaboration with RPMI. The RR in the 29 patients with metastatic colorectal carcinoma was 48% (28).

A different regimen piloted at the Hôpital Paul-Brousse in Villejuif, France, combined folinic acid at a dosage of 200 mg/m^2/day for 5 days by iv bolus followed immediately by 5-FU at 340–400 mg/m^2/day iv over 15 minutes for 5 days, with courses repeated at 21-day

TABLE 2.—Phase I/II trials of 5-FU + folinic acid in colorectal carcinoma

Institution	Investigator (ref No.)	Dosage		No. of patients (No. evaluable)	Prior treatment	Response
		Folinic acid	5-FU			
Mt. Sinai Hospital	Bruckner et al (26)	25–200 mg iv over 10 mins 1 hr prior to 5-FU every 21 days	25–35 mg/kg by iv bolus (total, 925–1300 mg/m^2)	32	32, none	1 CR, 6 PRs
RPMI	Madajewicz et al (27)	500 mg/m^2/wk × 6 as 2-hr infusion	300–750 mg/m^2/wk × 6 by bolus at midpoint of folinic acid infusion	26 (23)	11, none 12, 5-FU	3 PRs 6 PRs
Cleveland Metropolitan General Hospital and RPMI	Zakem et al (28)	500 mg/m^2/wk × 6 over 2 hrs	600 mg/m^2/wk × 6 by bolus at midpoint of folinic acid infusion	29	15, none 14, 5-FU	2 CRs, 12 PRs
Hôpital Paul-Brousse, Villejuif	Machover et al (29)	200 mg/m^2/day × 5 by bolus over 15 mins every 21 days	340–400 mg/m^2/day × 5	86	34, none 32, 5-FU	2 CRs, 19 PRs 2 CRs, 5 PRs
Vincent T. Lombardi Cancer Research Center and The Netherlands Cancer Institute	Byrne et al (30)	200 mg/m^2/day × 5 immediately after 5-FU every 28 days	350–400 mg/m^2/day × 5	43	23, none 19, 5-FU	5 PRs 0 PR
SWOG 8305	Budd et al (31)	200 mg/m^2/day × 4 by bolus every 28 days	1000 mg/m^2/day × 4 by CI	60 (53)	53, none	2 CRs, 10 PRs
		or				
		200 mg/m^2/day × 5 by bolus every 28 days	375 mg/m^2/day × 5 by bolus	61 (54)	54, none	3 CRs, 9 PRs
City of Hope	Bertrand et al (32)	500 mg/m^2/day × 6 by CI every 28 days	250–370 mg/m^2/on days 2–6 by bolus	36 (35)	35, 5-FU	3 PRs
RPMI	Herrera-Ornelas et al (33)	500 mg/m^2/day × 5 by CI	500 mg/m^2/day × 5 by CI	7	NA	0 PR
Cleveland Clinic	Cunningham et al (34)	60 mg/m^2/day × 5 by bolus prior to 5-FU every 28 days	7–12 mg/kg/day × 5 by bolus (260–444 mg/m^2/day × 5)	51 (36)	12, none 11, 5-FU	1 CR, 6 PRs 0 CR, 3 PRs
		or				
		60 mg/m^2/day × 4 by bolus prior to 5-FU every 28 days	600–1200 mg/m^2/day × 4 by CI		13, none	4 PRs
University of Washington	Mortimer and Higano (35)	60 mg/m^2 by bolus every 6 hrs × 4 doses every 28 days	600–800 mg/m^2/day × 4 by CI	23 (20)	16, no 5-FU 4, 5-FU	2 PRs 1 PR
Albert Einstein Medical Center	Greene et al (36)	60 mg/m^2/day × 4 by bolus every 28 days	400 mg/m^2/day × 4 by CI	25	10, none 15, 5-FU	2 PRs 0 PR
University of California, San Francisco, and Veterans Administration Medical Center	Lopez et al (37)	20–30 mg/m^2/wk × 3–4 by bolus 30 mins after 5-FU every 21–28 days	400–850 mg/m^2/wk × 3–4 by bolus	7	2, chemotherapy 3, radiation	4 PRs
Hannover Medical School	Schmoll and LeBlanc (38)	200 mg/m^2 over 10 mins every 21 days	30–40 mg/kg by bolus (1100–1400 mg/m^2) 50 mins after folinic acid	68	68, none	12 PRs
Swedish Hospital	Kaplan and Rivkin (39)	50 mg/m^2/day × 5 by bolus	400 mg/m^2/day × 5 by bolus after folinic acid	16	16, 5-FU	0 PR

intervals (41). The investigators (12) stated that the target plasma concentration of folinic acid was 10 μM, the level required for maximal potentiation of fluoropyrimidine toxicity in cell culture. The most prominent toxicity was oral mucositis; myelosuppression, diarrhea, and mild nausea were also observed. Two CRs and 19 PRs (RR, 39%) were seen in 54 patients with metastatic colorectal carcinoma who had not been previously treated with chemotherapy (29). Fewer responses were seen in the 32 patients who had received 5-FU either alone or in combination with other agents: two CRs and five PRs (RR, 22%). The median time to disease progression for the responding patients was 10 months (range, 2–41). Pharmacologic monitoring was reported in six patients, using both HPLC and microbiologic assays. The mean peak plasma concentration of the *l*-5-CHO-FH_4 was 43 μM, and a concentration > 10 μM was maintained for approximately 1 hour (table 1). There was no direct evidence, however, that comparable levels of intracellular reduced folates were achieved.

Two other studies utilizing the Villejuif regimen have been conducted. The interval between treatments, however, was 28 days rather than 21 days (30,31). Byrne et al (30) observed five PRs (22%) in 23 patients with no prior cytotoxic therapy, with a median duration of response of 10 months (range, 8–17). No responses occurred in patients with prior chemotherapy. Budd et al (31) reported a 22% response rate in 54 previously untreated patients.

Bertrand et al (32) at the City of Hope (Duarte, CA) used an alternate schedule featuring a CI of folinic acid at a dosage of 500 mg/m^2/day for 6 days in an effort to achieve high Cp_{ss} of biologically active reduced folates. Twenty-four hours after the start of the infusion, 5-FU was given at 250–370 mg/m^2/day for 5 days by iv bolus. Thirty-six patients who were reported to have unequivocal evidence of disease progression while receiving fluoropyrimidines were treated, and three PRs (9%) were seen, with durations of 4.3–5.4 months. The dose-limiting toxic effects were leukopenia (granulocytopenia) and mucositis. Pharmacokinetic studies determined that the Cp_{ss} of *l*-5-CHO-FH_4 and 5-CH_3-FH_4 were 4.5 ± 0.5 and 4.7 ± 0.9 μM, respectively (42). The investigators concluded that sustained levels of bioactive folates approaching 10 μM could be achieved by CI of high-dose folinic acid. In contrast, a preliminary report from RPMI of the pharmacokinetics of folinic acid (500 mg/m^2) plus 5-FU (500 mg/m^2) with both drugs given by CI for 5 days indicated that the steady-state Cp_{ss} of *l*-5-CHO-FH_4 was approximately 0.6 μM (33).

Several other trials have been conducted employing lower doses of folinic acid in combination with 5-FU either by bolus daily for 5 days or by CI daily for 4 days (34–39). For example, 36 evaluable patients with colorectal carcinoma were entered in a phase I/II trial at the Cleveland Clinic with 5-FU administered either at 300–444 mg/m^2/day for 5 days by bolus or at 600–1200 mg/m^2/day for 4 days by CI; folinic acid was administered at 60 mg/m^2/day as a bolus prior to 5-FU (34). The overall RR in 13 patients with no prior chemotherapy treated with the infusion schedule was 30% (four patients), with all responses occurring at dosages $\geq$ 100 mg/m^2/day. On the bolus schedule, responses were observed at doses $\geq$ 300 mg/m^2, with an overall RR of 58% (seven of 12 patients) in those with no prior therapy. The median duration of response was 4 months (range, 1.5–7+). Mucositis and myelosuppression were dose-limiting for the intermittent schedule, with a recommended phase II dosage of 5-FU of 370 mg/m^2/day for 5 days plus folinic acid at 60 mg/m^2/day for 5 days. Mucositis was dose-limiting on the CI schedule, with a recommended phase II 5-FU dosage of 1000 mg/m^2/day for 4 days plus folinic acid at 60 mg/m^2/day for 4 days. Other gastrointestinal toxic effects included diarrhea, proctitis, and nausea and vomiting. Pharmacologic monitoring of reduced folate levels was not performed.

In summary, a variety of schedules of folinic acid and 5-FU were studied in the phase I/II setting. Responses were seen in patients previously exposed to single-agent 5-FU; some of these patients had progressive disease while receiving single-agent 5-FU. The response rates in general were higher in patients with no prior 5-FU exposure. No individual regimen appeared to be clearly superior to the others on the basis of these phase II studies involving relatively small numbers of patients. Pharmacologic monitoring was carried out in several studies; the results suggest that plasma levels of reduced folates comparable to the level associated with enhancement of 5-FU cytotoxicity in vitro could be attained in patients.

PHASE III STUDIES IN COLORECTAL CANCER

Based on the phase II data suggesting activity of 5-FU given in combination with folinic acid, six phase III studies in metastatic colorectal carcinoma have been initiated to precisely define any increase in efficacy for the combination over standard therapy with 5-FU alone (table 3). Erlichman et al (43) at the Princess Margaret Hospital in Toronto are comparing the Villejuif regimen with 5-FU administered alone at an equivalent starting dose in previously untreated patients with metastatic colorectal carcinoma. Doses of 5-FU are escalated in subsequent courses to maintain equal toxicity. A preliminary analysis of 58 evaluable patients indicated that ten responses were observed in the group receiving folinic acid plus 5-FU versus none in the group receiving 5-FU alone. The median number of treatment courses for 5-FU alone was two; the mean 5-FU dosage had increased to 440 mg/m^2/day for 5 days by the fourth course, at which point the incidences of stomatitis, diarrhea, and neutropenia were comparable between the groups receiving 5-FU alone or folinic acid plus 5-FU. A total of 130 patients have been entered in the study, and a final analysis with a minimum of 1 year of follow-up after completion of treatment will be available in the near future.

TABLE 3.—Phase III trials of 5-FU + folinic acid in colorectal carcinoma

Institution	Investigators (ref No.)	Dosage		No. of patients	Response
		Folinic acid	Drug		
Princess Margaret Hospital	Erlichman et al (43)	200 mg/m^2/day × 5 by bolus prior to 5-FU	5-FU at 370 mg/m^2/day × 5 by bolus every 28 days	34	10 CRs + PRs
			vs		
		None	370 mg/m^2/day × 5; escalated to 440 mg/m^2/day × 5 as tolerated	24	0 CR + PR
NCOG	Valone et al (44,53)	200 mg/m^2/day × 5 by bolus	5-FU at 400 mg/m^2/day × 5 by bolus every 28 days	Target: 350	Coded
			vs		
		None	Loading course, 12 mg/kg/day × 5 (440 mg/m^2/day × 5); maintenance, 15 mg/kg/wk (550 mg/m^2/wk)		Coded
			vs		
		10 mg/m^2 every 6 hrs × 8 given 24 hrs after MTX for rescue	MTX at 50 mg/m^2 orally every 6 hrs × 5; 5-FU at 500 mg/m^2 24 hrs after MTX		Coded
North Central Cancer Treatment Group	O'Connell et al (unpublished data)	200 mg/m^2/day × 5	5-FU at 370 mg/m^2/day × 5 by bolus	Target: 420	Coded
			vs		
		20 mg/m^2/day × 5	5-FU at 425 mg/m^2/day × 5 by bolus		Coded
			vs		
		None	5-FU at 500 mg/m^2/day × 5 by bolus		Coded
			vs		
		15 mg/m^2 orally × 8 doses beginning 24 hrs after start of MTX infusion	MTX at 200 mg/m^2 as 4-hr infusion; then 5-FU at 1000 mg/m^2 by bolus 7 hrs after MTX		Coded
			vs		
		None	MTX at 40 mg/m^2 by bolus; then 5-FU at 700 mg/m^2 by bolus 24 hrs after MTX		Coded
			vs		
		None	5-FU at 300 mg/m^2 by bolus; then cisplatin at 20 mg/m^2 as 2-hr infusion		Coded
RPMI	Petrelli et al (45)	500 mg/m^2/wk × 6 as 2-hr infusion	5-FU at 600 mg/m^2/wk × 6 at midpoint of folinic acid infusion; rest for 2 wks; then repeated	25	1 CR, 11 PRs

continued

TABLE 3.—*continued*

Institution	Investigators (ref No.)	Dosage		No. of patients	Response
		Folinic acid	Drug		
			vs		
		None	5-FU at 450 mg/m^2/day × 5; then 200 mg/m^2 every other day × 6 doses by bolus; repeated every 6 wks	19	2 PRs
			vs		
		None	MTX at 50 mg/m^2/wk × 4 as 4-hr infusion followed by 5-FU at 600 mg/m^2/wk × 4, then every 2 wks	22	2 PRs
GITSG	Petrelli et al (unpublished data)	500 mg/m^2/wk × 6	5-FU at 600 mg/m^2/wk × 6 by bolus	Target: 450	Coded
			vs		
		25 mg/m^2/wk × 6	600 mg/m^2/wk × 6 by bolus		Coded
			vs		
		None	500 mg/m^2/day × 5 by bolus every 4 wks		Coded
City of Hope	Doroshow et al (49)	500 mg/m^2/day × 6 by CI	5-FU at 370 mg/m^2/day × 5 by bolus beginning 24 hrs after folinic acid, every 28 days	29	1 CR, 12 PRs
			vs		
		None	370 mg/m^2/day × 5 by bolus every 28 days	34	5 PRs

The Northern California Oncology Group (NCOG) is comparing the Villejuif regimen with single-agent 5-FU administered as a loading course at 440 mg/m^2/day for 5 days, followed by a weekly dose of 550 mg/m^2, versus sequential methotrexate (MTX)/5-FU in patients with colorectal carcinoma. Patients with prior 5-FU or fluorodeoxyuridine exposure were randomized to either 5-FU/folinic acid or MTX/5-FU. Only one PR has been observed in 40 evaluable patients with prior therapy who received 5-FU/folinic acid, and one CR has occurred in 42 previously treated patients who received MTX/5-FU (44). The results in patients with no prior therapy are not yet available, but the accrual goal of 350 patients has been reached.

The North Central Cancer Treatment Group is conducting a six-arm randomized phase III trial comparing both low-dose and high-dose folinic acid/5-FU versus 5-FU alone (at 500 mg/m^2/day × 5) versus two schedules of sequential MTX/5-FU versus 5-FU/cisplatin. The study has a target accrual of 420 patients (70 patients per arm). The results of this study are still coded.

A three-arm phase III study evaluating 5-FU/folinic acid versus 5-FU alone versus sequential MTX/5-FU has been conducted at RPMI in patients with previously untreated colorectal carcinoma (45). The schedule of single-agent 5-FU is not directly comparable to that of 5-FU/folinic acid. Single-agent 5-FU is given as a loading course every 6 weeks (450 mg/m^2/day × 5 by iv bolus, followed by 200 mg/m^2 every other day for six doses). Although higher loading doses of 5-FU have been employed, they have been associated with a significant incidence of stomatitis and myelosuppression (46). The 5-FU has been given at dosages up to 550 mg/m^2/day for 5 days, then in half-doses every other day for four to 11 doses or until toxicity occurred. Therefore, the dose in the 5-FU arm is the maximally tolerated dose. A preliminary report indicated that one CR and 11 PRs (48%) had been seen in 25 evaluable patients randomized to 5-FU/folinic acid versus two PRs (10%) in 19 evaluable patients randomized to 5-FU (43).

The Gastrointestinal Tumor Study Group (GITSG) is performing a three-arm phase III trial comparing the regimen of high-dose folinic acid/5-FU given weekly for 6 weeks to a low-dose regimen versus 5-FU alone administered as a bolus at 500 mg/m^2/day for 5 days. The study has a target accrual of approximately 150 patients per arm, which has been reached; the results are still coded.

The City of Hope is also conducting a phase III study comparing high-dose folinic acid as a CI plus bolus 5-FU versus 5-FU alone. The study design utilizes the same dose of 5-FU in both treatment arms, rather than using a higher equitoxic dose of 5-FU in the control arm. A recent update indicated that the response rate was 45% (one CR and 12 PRs in 29 patients) in the 5-FU/folinic acid arm versus 15% (five PRs in 34 patients) in the arm receiving 5-FU alone (47). Although the median time to disease progression was longer in the 5-FU/folinic acid arm (8 vs 3.9 mos), no difference in overall survival was seen.

FOLINIC ACID PLUS 5-FU COMBINATIONS IN OTHER MALIGNANCIES

Phase II studies of 5-FU/folinic acid have also been conducted in patients with other gastrointestinal adenocarcinomas, breast carcinoma, and squamous cell carcinoma of the head and neck (table 4). Machover et al (29) studied 27 evaluable patients with advanced gastric carcinoma in their phase II trial of 5-FU/folinic acid. One CR and 12 PRs (50%) were seen in 26 patients with no prior chemotherapy. The median time to disease progression of responders was 5.5 months (range, 2.1–28.7). The Southwest Oncology Group (SWOG) is conducting a randomized phase II study of two schedules of 5-FU plus folinic acid, including the schedule of bolus daily for 5 days, in patients with gastric carcinoma who have had no prior chemotherapy (SWOG 8390). Arbuck et al (48) treated 22 patients with gastric carcinoma with the RPMI weekly regimen. One PR was seen in ten patients with no prior chemotherapy (10%), and two PRs occurred in 12 previously treated patients (9%).

The City of Hope (49) has reported four PRs in 24 patients (17%) with metastatic breast carcinoma who received high-dose folinic acid by CI in combination with bolus 5-FU; the majority of these patients had received prior chemotherapy.

Twenty-eight women with metastatic breast carcinoma, 27 of whom were previously treated, received 5-FU/folinic acid in Brescia, Italy, according to the

TABLE 4.—Phase II trials of 5-FU + folinic acid in other malignancies

Institution	Investigator (ref No.)	Folinic acid	5-FU	No. of patients (No. evaluable)	Prior treatment	Response
			Gastric carcinoma			
Hôpital Paul-Brousse	Machover et al (29)	200 mg/m^2/day × 5 by bolus	340–400 mg/m^2/day × 5 over 15 mins every 21–28 days	27	26, none 1, chemotherapy	1 CR, 12 PRs 0 CR + PR
RPMI	Arbuck et al (48)	500 mg/m^2/wk × 6 as 2-hr infusion	600 mg/m^2/wk × 6 at midpoint of infusion	28 (25)	13, none 12, chemotherapy	1 PR 2 PRs
SWOG 8390	Berenberg et al (study not complete)	200 mg/m^2/day × 4 by bolus	1000 mg/m^2/day × 4 by CI	Target: 40	None	NA
			or			
		200 mg/m^2/day × 5 by bolus	375 mg/m^2/day × 5 by bolus	Target: 40	None	NA
			Breast carcinoma			
City of Hope	Doroshow et al (49)	500 mg/m^2/day × 6 by CI	370 mg/m^2/day × 5 by bolus starting 24 hrs after folinic acid, every 28 days	24	20, prior anthracycline 18, ≥ 2 prior regimens	4 PRs
University of Brescia	Marini et al (50)	200 mg/m^2/day × 5 by bolus	340–400 mg/m^2/day × 5 over 15 mins every 28 days	28	1, none 27, chemotherapy	3 CRs, 14 PRs
National Cancer Institute	Allegra et al (unpublished data)	500 mg/m^2/day × 5 by bolus	370 mg/m^2/day × 5 by bolus 2 hrs after folinic acid, every 28 days	NA	≥ 2 prior regimens	NA
			Head and neck carcinoma			
Mt. Sinai Hospital	Bruckner et al (25)	25 mg by iv bolus	22.5–35 mg/kg by bolus (925–1300 mg/m^2) every 21 days	16	NA	3 PRs
			Pancreatic carcinoma, unknown primary tumor			
Mt. Sinai Hospital	Bruckner et al (51)	200 mg by iv bolus	30 mg/kg by iv bolus every 14–21 days (1100 mg/m^2)	8	NA	1 CR, 3 PRs

Villejuif regimen of daily administration for 5 days. There were three CRs and 14 PRs (61%), with a mean response duration of 6 months (range, 2–9) (50). Myelosuppression was mild; only one patient experienced a platelet count $< 50{,}000/mm^3$ and none had a granulocyte count $< 1000/mm^3$. However, severe mucositis developed in 46%, severe diarrhea occurred in 57%, and conjunctivitis was seen in 46%. Canadian investigators are also performing a phase II study of 5-FU/folinic acid on the schedule of daily treatment for 5 days in breast carcinoma patients with no prior therapy for metastatic disease. Results are not yet available from this study.

A study of patients with breast carcinoma treated with high-dose folinic acid (500 mg/m^2/day iv over 30 mins for 5 days) with 5-FU (375 mg/m^2/day × 5 by iv bolus 60 mins after the folinic acid infusion) is being conducted by the Medicine and Clinical Pharmacology Branches of the National Cancer Institute. All of the patients have been heavily pretreated. The results of this ongoing study have not yet been published, although preliminary results indicate that responses have been seen in these patients who have had extensive prior chemotherapy and prior 5-FU exposure (C. J. Allegra, personal communication, Mar 1987). A phase II study of 5-FU/folinic acid on the weekly high-dose schedule in patients with breast cancer is under way at the RPMI.

Bruckner et al (25) treated 16 patients with head and neck tumors with low-dose folinic acid/5-FU, and three PRs (19%) of brief duration were seen. Bruckner et al (51) have also presented preliminary results in patients with unknown primary or pancreatic carcinoma treated with a higher dose of folinic acid (200 mg) plus 5-FU. One CR and three PRs (50%) have been reported in the first eight patients treated with this regimen (51).

COMPARISON OF TOXIC EFFECTS

A summary of the toxic effects observed with treatment given weekly for 6 weeks or daily for 5 days and with the CI regimens of 5-FU plus folinic acid is presented in table 5. In three studies (25,28,48) using the RPMI weekly regimen (two in colorectal carcinoma and one in gastric carcinoma), the dose-limiting toxic effects were leukopenia (7.5% with wbc count nadir $< 2000/mm^3$) and diarrhea (46% overall and 13% severe). Severe stomatitis was unusual (1.5%). Equal numbers of previously treated patients and those with no prior cytotoxic therapy were entered in these studies. For comparison, Ansfield et al (46) tabulated the toxic effects

TABLE 5.—Toxic effects of 5-FU + folinic according to dosage*

	RPMI (refs 27,28,48)	Hôpital Paul Brousse (ref 29)	City of Hope (ref 32)
Dosage			
5-FU	600 mg/m^2/wk × 6 by bolus	370 mg/m^2/day × 5 by bolus	370 mg/m^2/day × 5 by bolus
Folinic acid	500 mg/m^2/wk × 6 over 2 hrs	200 mg/m^2/day × 5 by bolus	500 mg/m^2/day × 6 by CI
No. of patients	69	111	25
No prior treatment	23	80	0
Prior treatment	46	31	25
Toxic effects			
Hematologic			
Wbc count nadir ($cells/mm^3$)			
≥ 2000	64 (93%)	NA	18 (72%)
1000–1999	4 (6%)	NA	7 (28%)
< 1000	1 (1.5%)	2 (2%)	
Granulocyte count nadir ($cells/mm^3$)			
< 1000	NA	NA	16 (64%)
Platelet count nadir ($cells/mm^3$)			
≥ 50,000	69 (100%)	111 (100%)	24 (96%)
< 50,000	0	0	4 (4%)
Nonhematologic			
Diarrhea			
Mild to moderate	23 (33%)	25 (23%)	NA
Severe	9 (13%)	5 (4%)	0
Stomatitis			
Mild to moderate	NA	30 (27%)	23 (92%)
Severe	1 (1.5%)	3 (3%)	2 (8%)
Dry skin rash	8/40 (20%)	10 (9%)	0
Conjunctivitis	23 (33%)	12 (11%)	0
Alopecia	2/18 (11%)	12 (11%)	NA

*Unless otherwise specified, values = No. of patients.

seen in 90 patients who received single-agent 5-FU at a dose of 555 mg/m^2/week (not to exceed 1000 mg): 4% had a wbc count nadir $< 2000/mm^3$, 41% had diarrhea (6% severe), and 1% had severe stomatitis. The toxicity profile seen with 5-FU alone is similar to that noted in the studies of weekly 5-FU/folinic acid.

Fifty-two percent of the patients in the 5-FU/folinic acid arm of the phase III trial at RPMI, all without prior chemotherapy, experienced dose-limiting diarrhea (45). Eight of these 13 patients required hospitalization for iv hydration, and there was one treatment-related death. Severe gastrointestinal toxicity characterized by diarrhea (with or without nausea and vomiting) with dehydration has also been observed in both the low-dose and high-dose folinic acid/5-FU arms in the GITSG phase III trial (52).

In contrast, Machover et al (29) reported that oral mucositis was the dose-limiting toxicity on the 5-FU/folinic acid schedule of daily treatment for 5 days, with an overall incidence of 30%. This study included patients with colon and gastric carcinoma; approximately 70% had not received prior chemotherapy. Other investigators using the same regimen have also reported a high incidence of stomatitis: 22% in the SWOG phase II trial in colorectal carcinoma (31); 20% in the NCOG phase III trial in colorectal carcinoma (53); and 46% in the Brescia trial for breast carcinoma (50). A 22% incidence of severe or worse myelosuppression (wbc count nadir $< 2000/mm^3$ or platelet count nadir $< 50{,}000$) was also noted in the SWOG study, and 16% of the patients developed skin rashes (31). In comparison, Seifert et al (54) described the toxic effects in patients who received 5-FU at 450 mg/m^2/day for 5 days: stomatitis, 88% overall (16% severe); diarrhea, 31%; and dermatitis, 16%.

The regimen employing 5-FU given by bolus daily for 5 days in combination with high-dose CI folinic acid in previously treated patients with colorectal carcinoma was associated with dose-limiting granulocytopenia in two-thirds of the patients (32). Stomatitis occurred in all patients. The granulocytopenia observed in this study is higher than might be expected with 5-FU alone given by bolus daily for 5 days (54). It is difficult to draw a conclusion as to whether folinic acid is definitely modulating the toxicity of 5-FU on the basis of the phase II results. Two of the phase III studies (City of Hope and Princess Margaret Hospital) are comparing an equal dose of 5-FU with or without folinic acid for at least the first course. A comparison of the toxic effects observed in each arm should provide more conclusive evidence regarding the modulation of 5-FU toxicity by folinic acid in normal host tissues. Preliminary analysis of the City of Hope trial indicates that stomatitis is more severe in the 5-FU/folinic acid arm (47).

BIOCHEMICAL CORRELATES IN HUMAN TISSUE

At present, there are limited data about the degree of TS inhibition in humans. Spears et al (55) reported the degree of TS inhibition in biopsies of solid tumor and normal liver obtained 20–240 minutes following administration of 5-FU at a dose of 500 mg/m^2 in 21 patients who were undergoing an elective surgical procedure. Thirteen had colorectal carcinoma, six had other gastrointestinal malignancies, and two had breast carcinoma. Maximal TS inhibition occurred within 90 minutes and averaged 70%–80% in tumor biopsies, compared to approximately 50% inhibition in histologically normal liver. Since samples were not taken after 4 hours, the duration of TS inhibition was not ascertained. Rustum et al (56) presented preliminary data on five patients who had > 50 g of tumor tissue removed at various times following a 2-hour infusion of folinic acid (500 mg/m^2) in combination with 5-FU (600 mg/m^2). Compared to baseline activity, the activity of TS was inhibited by 60%–85% at 2–8 hours following administration of 5-FU/folinic acid. Three patients had additional samples drawn 22 hours after dosing. The TS activity had returned to baseline in two patients but remained 67% inhibited in the third patient.

Allegra et al (personal communication, Mar 1987) at the National Cancer Institute, in collaboration with investigators at the Mayo Clinic, are obtaining sequential biopsies in patients with breast carcinoma participating in their phase II trial of 5-FU/folinic acid. Patients with accessible tumor tissue have a baseline biopsy, and sequential biopsies are taken 6 and 24 hours following a dose of 375 mg/m^2 of 5-FU alone. Biopsies are repeated following the same dose of 5-FU in combination with folinic acid at 500 mg/m^2. Using a [^{3}H]FdUMP assay, the total [^{3}H]FdUMP and the percents bound and unbound are calculated. Each patient thus serves as her own control. The kinetics of TS-FdUMP complex formation will be compared with treatment outcome. Following completion of the initial phase, the study will be modified so that patients will receive 5-FU at 500 mg/m^2 alone on Day 1, followed by 5-FU at 375 mg/m^2 in combination with folinic acid on Days 2–5, with biopsies obtained at comparable times. This will permit a direct comparison of the degree and duration of ternary complex formation following a maximally tolerated dose of 5-FU alone given daily for 5 days.

DISCUSSION

Various doses and schedules of 5-FU in combination with folinic acid have produced responses in patients who had previously had treatment failure with 5-FU alone. The combination has been associated with RR of 20%–50% in previously untreated patients. A note of caution might be interjected before accepting the efficacy of any 5-FU/folinic acid regimen prior to completion of the definitive phase III trials. Confidence intervals at the 95% significance level can be calculated by application of the following equation: $1.96 \times {}^{1/2}[(p \times q)/n]$, where p is the response rate, $q = 1 - p$, and n is the number of patients (57). For example, the trial reported by Machover et al (29) included 86 patients with colorectal carcinoma. The overall RR was 33% (95% confidence interval, 23%–43%) for all patients and 39% (26%–

52%) in previously untreated patients. An RR of 22% (95% confidence interval, 11%–33%) was reported for the arm of the SWOG study (54 evaluable patients) treated with the Machover regimen (31). Although the response rates appear to be different, the confidence intervals overlap. Machover et al concluded that the results were promising, while Budd et al (31) viewed the SWOG data more conservatively. The weekly regimen piloted at RPMI, at first review, appears to be the most active of the 5-FU/folinic acid regimens. The RRs in the trials reported by Madajewicz et al (27) and Zakem et al (28) were 39% (95% confidence interval, 24%–54%) and 48% (30%–66%), respectively. Again, the confidence intervals overlap with those of the schedule for daily administration by bolus for 5 days. Therefore, although the phase II trials can screen a regimen for activity, the breadth of the confidence intervals suggests that the observed response rates cannot be taken as precise indicators of the regimen's efficacy.

A number of questions have not yet been answered. What is the optimal regimen? Two of the ongoing phase III trials are comparing high-dose versus low-dose folinic acid plus 5-FU given on a particular schedule. None of the phase III trials compare the three major schedules of 5-FU/folinic acid. A prospective randomized trial comparing the 5-FU/folinic acid regimens would be necessary to ascertain if any of the regimens were superior, and up to 500 patients per arm might be required to detect a 10 percentage point superiority of one arm with 90% power. It is doubtful if such a trial would generate much enthusiasm or be a good use of patient resources. The doses of folinic acid chosen for a given regimen were selected empirically. Although pharmacologic data suggest that each of these doses can produce the theoretical target plasma concentration of reduced folates, the "optimal dose" of folinic acid for a fixed maximally tolerated dose of 5-FU has not been determined. A response surface design might permit comparison of responses across a spectrum of folinic acid doses with a constant 5-FU dose (58). The cumulative data points across the continuing variable (ie, the folinic acid dose) could provide adequate power to select a "best" folinic acid dose with fewer patients than would be required for a direct comparison of a low, medium, and high dose on a fixed schedule. Disparate schedules such as weekly for 5 weeks versus daily for 5 days, however, could not be compared in the same trial.

Does the combination of 5-FU and folinic acid in patients result in greater inhibition of TS than that achieved at maximal doses of 5-FU alone? If so, is such potentiation selective for malignant tissue as opposed to normal tissue? Is there a correlation between the effect of folinic acid in combination with 5-FU on the duration of inhibition of TS and the clinical response to treatment? Current and future clinical studies that incorporate biochemical end points may provide such crucial information. In another setting, biochemical determinants of response to 5-FU will be measured in the primary tumor tissue obtained at surgery in two National Surgical Adjuvant Breast and Bowel Project (NSABP) adjuvant colorectal trials that are under development and incorporate a 5-FU/folinic acid arm. The cellular pharmacologic studies planned include measurement of 5,10-CH_2-FH_4 pools; endogenous TS activity; FdUMP-TS complex formation in situ; total intracellular folate pools; and the activities of several enzymes involved in the anabolism of 5-FU to its active nucleotides. This long-term prospective study may provide important information on whether the initial biochemical profile of tumor tissue can predict clinical outcome.

Another issue is how the 5-FU/folinic acid strategy fits in with other pharmacologic strategies to enhance the efficacy of 5-FU therapy. The vast preclinical literature on 5-FU suggests that it may exert cytotoxicity through multiple mechanisms. Unfortunately, there are fewer data providing conclusive information on the mechanisms of resistance in patients. The levels of FUMP and FUTP formed have correlated with the toxicity of 5-FU in several preclinical models, suggesting that the RNA-mediated effects of 5-FU are the critical mechanisms for toxicity (fig 1) (1–3,59,60). A variety of other agents including MTX, thymidine, *N*-phosphonacetyl-L-aspartic acid, and 6-methylmercaptopurine riboside have also been shown in vitro and in vivo to modulate 5-FU cytotoxicity by enhancing the incorporation of 5-FU into RNA (61–65). A number of these preclinical observations have been carried forward into clinical studies. Negative trials have been reported, but promising results have also been seen with rationally designed schedules (66–70).

Experimental models that have explored time-dose relationships for 5-FU cytotoxicity against human carcinoma cell lines in vitro have indicated that prolonged exposure to 5-FU at relatively low doses is more effective than short-term high-dose exposure in producing direct cytotoxic effects (71). Prolonged, low-dose exposure to 5-FU is thought to enhance the incorporation of FUTP into RNA, which may maximize the RNA-directed cytotoxic effects of 5-FU (72). Lokich (73) has recently reviewed the evidence suggesting that 5-day CI of 5-FU may be more efficacious than administration of 5-FU on a bolus schedule. The results of two prospective randomized trials were cited. Seifert et al (54) randomized 70 patients with metastatic colorectal carcinoma to bolus 5-FU (450 mg/m^2/day $\times$ 5) versus CI 5-FU (1100 mg/m^2/day $\times$ 5). The RR in the bolus group was 22%, with a median survival of 2 months; the RR was 44% in the CI group, with a median survival of 8 months. The toxicity profile differed in the two arms, with severe leukopenia and severe stomatitis reported in 30% and 16% of the patients, respectively, in the bolus arm, compared to absence of severe leukopenia but a 65% incidence of severe stomatitis in the CI arm. In a randomized trial by Kish et al (74), cisplatin (100 mg/m^2 iv on Day 1) was given in combination with either CI 5-FU (1000 mg/m^2/day $\times$ 4) or bolus 5-FU (600 mg/m^2 iv on Days 1 and 8) to patients with head and neck carcinoma. The RR was 20% for the 20 patients in the bolus

group (two CRs and two PRs) versus 72% for the 18 patients in the CI group (four CRs and nine PRs; $P = 0.01$). Again, the toxicity profile differed between the two schedules. More recently, a number of phase II studies of protracted venous infusion of 5-FU in patients with metastatic colorectal carcinoma have been conducted. Several investigators have reported favorable results, with response rates of 27%–44% (75–79). Prolonged infusion of 5-FU is being compared against a more conventional bolus schedule of 5-FU in a randomized phase III clinical trial of the Mid-Atlantic Oncology Program. Preliminary analysis indicates that the RR in the CI arm (30%) is superior to that in the bolus regimen (8%) (80).

The combination of cisplatin and 5-FU is also being explored in several carcinomas, including colorectal and head and neck carcinomas. Preclinical in vitro and in vivo models have demonstrated synergism between cisplatin and 5-FU (81–83). The mechanism for the synergism has not been completely defined but may in part be explained by the effect of cisplatin on intracellular folate pools. Cisplatin can inhibit the transport of the essential amino acid methionine (84). Cells deprived of exogenous methionine must then endogenously synthesize methionine from homocysteine to permit normal growth. This is accomplished by an increase in the conversion of 5-CH_3-FH_4 to FH_4 (fig 2). Cisplatin has been reported to increase the pools of FH_4 and 5,10-CH_2-FH_4, which subsequently can result in an increase in TS ternary complex formation (82). Results from a number of phase II studies have suggested a beneficial effect of 5-FU plus cisplatin in colorectal carcinoma and in head and neck carcinoma (74,85,86).

Thus, a variety of approaches to improve the activity of 5-FU are being studied, and it is premature to conclude that one approach is superior to another. Several of the phase III trials in advanced colorectal carcinoma outlined in table 3 include a comparison of sequential MTX/5-FU or cisplatin/5-FU with single-agent 5-FU and 5-FU/folinic acid. The results of these comparative trials should provide information on whether any of the combinations of 5-FU and other drugs that are mentioned here are superior to 5-FU or have any advantage relative to each other.

What is the spectrum of activity of 5-FU/folinic acid? Based on the phase II results in colorectal carcinoma, considerable enthusiasm for a broader application of the combination has been generated. Several phase II trials of the combination have been or are being conducted in tumors in which 5-FU has some single-agent activity, such as breast, gastric, pancreatic, and head and neck carcinoma. Further screening of the activity of 5-FU/folinic acid will not await determination of the optimal dose and schedule from colorectal carcinoma trials, since several years may be required to determine this. There is as yet no definitive answer as to which of the numerous 5-FU/folinic acid schedules has the greatest advantage in terms of antitumor efficacy or therapeutic index. There may be less impetus to study the activity of 5-FU/folinic acid in tumors considered insensitive to 5-FU alone, although phase II studies of the combination in non-small cell lung cancer and low-grade B-cell malignancies have been initiated.

Is there an advantage to regional administration of 5-FU/folinic acid? Phase I studies exploring ip administration of 5-FU/folinic acid have been conducted (87,88), and clinical trials of 5-FU/folinic acid given by hepatic artery infusion for liver metastases have been initiated. It is too early to comment on whether regional therapy with this combination will prove fruitful.

Another concept to consider is whether efforts should be directed toward developing combination regimens involving 5-FU/folinic acid. In colorectal carcinoma, the RR of 5-FU/folinic acid is $< 50\%$, and only a minority of patients have achieved CR. Additional agents may be selected on the basis of different mechanisms of action, lack of cross-resistance, and nonoverlapping toxic effects. For example, semustine given in combination with 5-FU with or without vincristine has modest activity in advanced colorectal carcinoma, and the results of the NSABP colon and rectal adjuvant studies C-01 and R-01 have indicated a benefit to some patients who received therapy with semustine, 5-FU, and vincristine (89–93). Phase I trials have been initiated to determine whether semustine can be added to 5-FU/folinic acid (given at full doses) on two different schedules with acceptable toxicity. A pilot study of weekly 5-FU/folinic acid in combination with low-dose cisplatin is under way at RPMI. A regimen for breast cancer could be evaluated for the feasibility of combining cyclophosphamide with or without MTX or doxorubicin with 5-FU/folinic acid.

Another issue that deserves comment is the cost of folinic acid for parenteral use. The direct cost of a 50-mg vial of folinic acid was approximately $78 in 1986, and the cost to the patient was higher. The expense for one course of folinic acid at 500 mg/m^2 weekly for 6 weeks is estimated to be a minimum of $4680. Two 3-week courses of folinic acid at 200 mg/m^2/day for 5 days might cost a minimum of $3120. Since the cost is considerable, it would be difficult to justify the expense of folinic acid for routine use unless the results of the phase III trials indicate that its addition to 5-FU results in a survival advantage and/or an improvement in the quality of life.

Is iv administration of folinic acid necessary to achieve adequate levels of reduced folates? Following oral administration of folinic acid, it is rapidly absorbed and enters the general body pool of reduced folates. Only small amounts of *l*-5-CHO-FH_4 escape presystemic metabolism by the gastrointestinal mucosa and/or the liver, and the increase in plasma folate activity is primarily due to 5-CH_3-FH_4 (18). Straw et al (18) demonstrated that following repeated oral administration of 25 mg of folinic acid every 8 hours, steady state was achieved by 64 hours, and the plasma concentration was approximately 1.5 μM. Absorption was saturable, however, and the values for area under the plasma concentration versus time curve following administration of 25, 50,

and 100 mg were comparable. McGuire et al (94) also reported nonlinear absorption of orally administered leucovorin. Recently, Hines et al (95) have reported that peak plasma levels of total reduced folates and *l*-5-CHO-FH_4 achieved following 800 mg of oral folinic acid were 4.8 ± 1.2 and 0.23 ± 0.11 μM, respectively. Based on these observations, the investigators explored treatment with high-dose oral folinic acid in combination with 5-FU in a phase I trial. Preliminary information indicates that following four hourly doses of leucovorin (total dose, 500 mg/m^2 orally), the total level of reduced folates was approximately 4 μM at the fifth hour (96). Clinical trials using high-dose oral leucovorin administered on a frequent basis in an effort to maintain adequate steady-state levels of reduced folates have been undertaken, but information from these trials is not yet available.

In summary, the combination of 5-FU/folinic acid has a sound biochemical basis. Encouraging results have been seen in colorectal and breast carcinoma. The phase III trials in advanced colorectal carcinoma will define the level of activity of the regimen.

Based on the results from phase II trials and preliminary data from the phase III trials, considerable enthusiasm for a broader application of the 5-FU/folinic acid combination has been generated. Since 5-FU is relatively well tolerated and is free of significant chronic toxicity, the National Cancer Institute of Canada has initiated an adjuvant study of 5-FU/folinic acid versus control in Dukes B_2 and C colon carcinoma following curative surgery. Plans have been made to incorporate 5-FU/folinic acid into the next adjuvant studies of colon and rectal carcinomas by the NSABP. The clinical experience thus far suggests that careful and rational application of strategies for biochemical modulation of 5-FU, which have been defined preclinically, may lead to novel approaches for improving the therapeutic efficacy and hopefully the therapeutic index of the antimetabolite. Because the use of exogenous reduced folates addresses only one mechanism of resistance to 5-FU, investigators should not be discouraged from pursuing alternative biochemical strategies to circumvent resistance due to other mechanisms. Innovative strategies will be required to develop combination regimens or alternating non-cross-resistant regimens that further improve on the modest results with the 5-FU/folinic acid combination.

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BRIEF REPORTS

Phase II Trial of rDNA Alfa 2b Interferon in Patients With Malignant Carcinoid Tumor[1,2]

David B. Smith,* J. Howard Scarffe, John Wagstaff, and Richard J. Johnston[3]

Fourteen patients with malignant carcinoid tumors were treated with rDNA alfa 2b interferon by sc injection three times per week. Treatment was started at a dose of 2 milliunits/m^2, with escalations to 3, 5, 7, and 10 milliunits/m^2 at 2-week intervals depending on toxicity. No objective tumor regressions were seen, but five of 14 patients (36%) had 50% reduction in 24-hour 5-hydroxyindoleacetic acid excretion and six of nine (67%) with carcinoid syndrome had a good symptomatic response. Biochemical responses occurred during the first 8 weeks of treatment, and escalation of the dose of interferon did not increase the response rate. The rDNA alfa 2b interferon has activity in patients with the carcinoid syndrome and should be used at a low dose (2-3 milliunits/m^2) until symptoms recur. [Cancer Treat Rep 71:1265-1266, 1987]

Malignant carcinoid tumors are a rare group of tumors accounting for 0.05%-2% of all neoplasms (1). These tumors have a relatively long natural history; median survival of untreated patients is approximately 2.5 years (2). Several chemotherapeutic agents have been tested in patients with malignant carcinoid tumors, but response rates are low, with little if any improvement in survival (3,4). In 1983, Öberg et al (5) reported that human leukocyte interferon could induce biochemical and symptomatic remissions in patients with carcinoid tumors, and thus the present study was initiated to test the effectiveness of rDNA alfa 2b interferon in this disease.

PATIENTS AND METHODS

All patients with a histological diagnosis of carcinoid tumor who were symptomatic and had a Karnofsky performance status of at least 40% were entered in the study. Pretreatment assessment included cbc, biochemical profile, liver function tests, chest x-ray, abdominal computerized tomography (CT) scan, and urinary 24-hour 5-hydroxyindoleacetic acid (5HIAA) excretion. Urinary 5HIAA determination was repeated every 2 weeks for 8 weeks and monthly thereafter. A follow-up CT scan was performed every 3 months.

Recombinant alfa 2b interferon (INTRON A) was supplied by Kirby-Warrick, Ltd (Mildenhall, Essex, England). The drug was administered by sc injection three times per week. Patients were started at a dose of 2 milliunits/m^2 per injection, with escalations to 3, 5, 7, and 10 milliunits/m^2 at 2-week intervals in the absence of dose-limiting toxicity (World Health Organization grade 3).

Three categories of response were assessed: (*a*) *objective*—standard World Health Organization criteria (6); (*b*) *biochemical*—partial remission, defined as a 50% decrease in 5HIAA excretion maintained for at least 4 weeks; (*c*) *symptomatic*—75% reduction in the number of episodes of flushing/diarrhea and control of pain.

RESULTS

Fourteen patients were entered in the study; patient characteristics are shown in table 1. All patients had elevated 24-hour urinary excretion of 5HIAA. Nine patients had symptoms of the carcinoid syndrome; five did not (abdominal pain, three; anorexia and weight loss, two). The median time from diagnosis of carcinoid tumor to the start of interferon therapy was 36 months (range, 2-192).

The median duration of interferon therapy was

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TABLE 1.—Patient characteristics*

Characteristic	
Total	14
Male:female ratio	8:6
Median age in yrs (range)	53 (33-76)
Karnofsky performance status	
50	4
60	5
70	3
80	2
Primary tumor site	
Midgut	11
Lung	2
Ovary	1
Prior chemotherapy	2
Sites of disease	
Liver	4
Liver + abdomen	8
Abdomen/pelvis	2
Symptoms	
Carcinoid syndrome	9
Flushing	6
Diarrhea	7
Abdominal pain	3
Anorexia + weight loss	2

*Unless otherwise specified, values = No. of patients.

14 weeks (range, 4–52); ten patients received at least 3 months of therapy. Reasons for stopping therapy included objective tumor progression (eight patients); interferon-related toxic effects, ie, anorexia and lethargy (four); and 1 year of treatment completed (one). One patient continues therapy at > 20 weeks.

Ten patients noted flu-like symptoms in the early stages of treatment, and ten developed varying degrees of lethargy as treatment progressed. Anorexia (two patients) and nausea (three) occurred less frequently. No hematologic toxicity was seen.

No objective tumor regressions occurred, as assessed clinically and by CT scan. However, in five (36%) of the patients, there was a > 50% decrease in 5HIAA excretion, and all five experienced significant relief of flushing and/or diarrhea. One additional patient had a symptomatic response in the absence of biochemical improvement, although the pretreatment 24-hour 5HIAA excretion was < 500 μmol, and this remained stable. These six patients showed an increase in Karnofsky performance status of at least 20. None of the patients with abdominal pain gained any symptomatic improvement from the therapy. There was no correlation between response and initial 5HIAA level, and in no case was there a 5HIAA response accompanied by objective tumor progression.

The five biochemical remissions occurred within 8 weeks of the start of therapy and were maintained for 8, 16, 16, 18, and 20 weeks. In all cases, interferon was discontinued due to lethargy. Symptomatic responses were evident after 4–6 weeks of treatment.

Median survival from diagnosis was 54 months (range, 5 mos to 17 yrs), and median survival from the start of therapy was 7 months (range, 2 to > 15).

DISCUSSION

No objective tumor regressions were seen during this study. However, five of the 14 patients (36%) had a > 50% reduction in 5HIAA excretion and six of nine (67%) had significant reduction in flushing and/or diarrhea. None of the patients with other symptoms experienced any relief. These results are similar to those recently updated by Öberg et al (7) for human leukocyte interferon. In that study of 36 patients, 47% achieved biochemical response and 64%, symptomatic response. These data suggest that the activity of rDNA alfa 2b interferon is similar to that of human leukocyte interferon in malignant carcinoid tumors.

There was no evidence that increasing the dose of interferon to > 3 milliunits/m^2 three times weekly improved symptomatic and biochemical remissions. All biochemical remissions occurred during the first 8 weeks of treatment. It thus appears that the correct way to use interferon in carcinoid tumors is to treat patients with low-dose therapy (2–3 milliunits/m^2 three times per week) and to continue for as long as the symptomatic response is maintained. Patients who respond, either biochemically or symptomatically, will do so within the first 4–8 weeks, and treatment should be stopped if no effect is seen by this time. Patients with symptoms other than those related to hormone production are unlikely to benefit from interferon therapy. A total of 60%–70% of patients with the carcinoid syndrome may experience symptomatic relief, and 30%–50%, biochemical response.

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BRIEF REPORTS

Interactions of Methotrexate and Cyclophosphamide With the Pharmacokinetics of 5-Fluorouracil in an Animal Model[1]

Ernst A. de Bruijn,* Oscar Driessen, Paul Leeflang, Elisabeth van Strijen, Nel van den Bosch, and Jo Hermans[2]

The interaction of methotrexate and/or cyclophosphamide with the pharmacokinetics of 5-fluorouracil (5-FU) was studied in tumor-bearing WAG/Rij rats. Four groups were formed including treatment with single-agent 5-FU (eight rats); 5-FU plus methotrexate (11 rats); 5-FU plus cyclophosphamide (12 rats); and 5-FU, cyclophosphamide, and methotrexate (13 rats). The area-under-the-plasma-concentration/time curve, total-body clearance, elimination half-life, mean residence time, and steady-state volume of distribution were computed and compared. The mean residence time and elimination half-life of 5-FU increased when methotrexate was included in the combination. The increase was significant ($P < 0.05$) for 5-FU, cyclophosphamide, and methotrexate versus 5-FU and cyclophosphamide. [Cancer Treat Rep 71:1267-1269, 1987]

The combination of cyclophosphamide, methotrexate, and 5-fluorouracil (5-FU) is a frequently applied regimen in cancer chemotherapy. In this regimen interactions at the pharmacokinetic level as expressed in changes in the total-body clearance (CL) of methotrexate have been described once (1). As a continuation of that paper we report here interactions of cyclophosphamide and methotrexate with 5-FU at the pharmacokinetic level indicating that this interaction is quantitatively important.

METHODS

The experiments described were the same as those extensively reported for methotrexate (1); in the present study, however, the influence of cyclophosphamide and methotrexate on 5-FU plasma concentrations is analyzed.

Animals

Female WAG/Rij rats (180-200 g) with transplanted rhabdomyosarcomas of about 1 cm^3 in both flanks were treated with single-agent 5-FU; 5-FU plus methotrexate (FM); 5-FU plus cyclophosphamide (FC); and 5-FU, cyclophosphamide, and methotrexate (FCM).

Study Design

This was a randomized study, in which at least eight rats were included per treatment group: 5-FU alone (eight rats), FM (11 rats); FC (12 rats); and FCM (13 rats). Cyclophosphamide (70 mg/kg) was administered orally by stomach tube and both methotrexate (2 mg/kg) and 5-FU (20 mg/kg) were administered by intra-arterial injection.

5-FU Plasma Concentrations

Blood samples were collected at 5, 15, 30, 60, 90, 120, 240, 360, and 480 minutes in 44 animals. Because of the short half-life of 5-FU, as a check blood was also collected in eight animals (two per treatment group) at the earlier time points of 1, 5, 15, 30, 45, 60, 75, 90, 105, and 120 minutes. The results obtained with these eight animals were consistent with the results obtained in the former groups, but are not included in the pharmacokinetic evaluations. Blood samples were centrifuged at $1000 \times g$ for 10 minutes and plasma was stored at $-35°C$ until analysis. 5-FU plasma concentrations were determined using gas chromatography with an assay sensitivity of 60 ng/ml.

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Computations

Pharmacokinetic calculations were carried out by linear regression analysis of the terminal parts of the plasma concentration-time curves elimination half-life and by noncompartmental analysis (AUC, CL = D/AUC), where AUC = area under the curve and D = dose. The mean residence time (MRT) and volume of distribution (V_{ss}) were calculated by moment analysis (1). For comparisons between all concentrations of all treatment groups over five time points the Friedmann two-way analysis of variance test was used. Analysis of pharmacokinetic data over the four groups was done using one-way analysis of variance (ANOVA), with Scheff's procedure for multiple comparisons. Statistical significance was determined at the 5% level.

RESULTS

Individual plasma concentrations together with mean concentrations per treatment group are presented in figure 1.

In comparing plasma concentrations of rats of different treatment groups over all time points, those of FCM-treated rats were significantly different from those of FC-treated rats (Friedmann two-way analysis of variance). Omission of the rather high concentrations of one FCM-treated rat in figure 1 did not alter the conclusions.

The mean CL was lowest in FCM-treated rats, which was related to an observed longest mean elimination half-life. Furthermore, an enhancement of the mean MRT was noted when methotrexate was included in the treatment schedule (FM- and FCM-treated rats). Prolongation of elimination half-life and MRT of 5-FU was most pronounced when FCM-treated rats were compared with FC-treated rats ($P = 0.01$ and $P = 0.002$, respectively; ANOVA).

DISCUSSION

This study observed that interactions of cyclophosphamide and methotrexate with 5-FU at the pharmacokinetic level occur. Methotrexate appeared to be capable of decreasing 5-FU clearance from 13.4 ml/minute

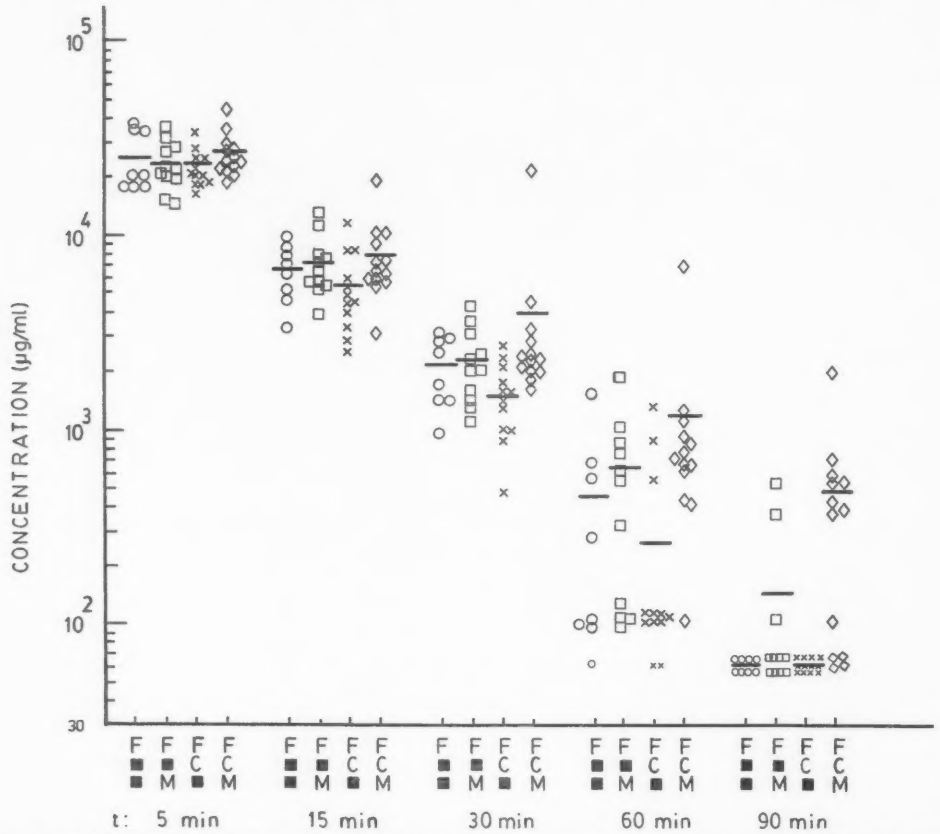

FIGURE 1.—Individual concentrations together with mean data of 5-FU in plasma of rats treated with 5-FU (○); FM (□); FC (X); and FCM (◇). Concentrations ≤ 60 ng/ml are indicated as 60 ng/ml.

TABLE 1.—Mean and SDs of pharmacokinetic parameters from 5-FU obtained for 5-FU-, FM-, FC-, and FCM-treated rats

	Treatment group			
	5-FU ($n = 8$)	FM ($n = 11$)	FC ($n = 12$)	FCM ($n = 13$)
AUC (ng/ml · min)	374 ± 114	365 ± 128	318 ± 93	442 ± 179
CL (ml/min)	11.6 ± 3.5	12.2 ± 3.8	13.5 ± 3.4	9.8 ± 2.7
$t_{½,z}$ (mins)*	9.8 ± 3.0	10.6 ± 3.7	7.8 ± 2.9	13.8 ± 4.9
MRT (mins)	12.6 ± 3.9	14.5 ± 4.8	10.1 ± 3.3	17.6 ± 5.5
V_{ss} (ml)	143 ± 45	171 ± 79	132 ± 41	167 ± 70

*$t_{½,z}$ = elimination half-life.

in FC-treated rats to 10.1 ml/minute in FCM-treated rats. It can be derived from figure 1 that cyclophosphamide tends to increase clearance (FC vs 5-FU; FM vs 5-FU). Consequently, 5-FU concentrations of FCM-treated rats are expected to be in between those of FM- and FC-treated rats and hence more like the 5-FU concentrations determined in singly 5-FU-treated rats. Surprisingly, addition of cyclophosphamide to the FM treatment results in an enhanced effect of methotrexate on 5-FU concentrations. Interactions of 5-FU and methotrexate at the biochemical level have often been described (2). Apparently, interactions at the pharmacokinetic level can also take place and might have impact on the effect since 5-FU clearance in FCM-treated rats appeared to be decreased by approximately 25% compared to FC-treated rats and by 16% compared to 5-FU-treated rats (table 1). Thus, in addition to interactions of 5-FU and cyclophosphamide with methotrexate pharmacokinetics (1), interactions of methotrexate with 5-FU pharmacokinetics also exist. These interactions might have implications for the outcome of (clinical) effect because 5-FU levels are maintained over prolonged periods, possibly resulting in an increase of cytotoxic effect. This will be evaluated in the same animals, scoring the effects on the tumor and bone marrow. Since 5-FU plasma concentrations are comparable to those observed in patients treated with the CMF regimen (3), similar pharmacokinetic interactions might occur. Hence, besides interactions of methotrexate with 5-FU at the cellular level (pharmacodynamic), pharmacokinetic interactions can take place and might be attributed to the efficacy of the combination of drugs in the CMF regimen (4,5).

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BRIEF REPORTS

Multiple Drug Intensification After Cyclophosphamide, Doxorubicin, and 5-Fluorouracil (CAF) Induction in Metastatic Breast Cancer: A Southeastern Cancer Study Group Phase II Trial[1,2]

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In earlier breast cancer clinical trials, the Southeastern Cancer Study Group (SECSG) identified "good-risk" and "poor-risk" patterns of metastatic breast cancer (MBC) (1) and subsequent controlled clinical trials were tailored to these subsets. In good-risk patients, levamisole was not found to improve on the results of cyclophosphamide, doxorubicin, and 5-fluorouracil (CAF) chemotherapy (2) and in poor-risk patients, a strategy of alternating combinations of drugs also did not provide significant improvement (3,4). The present trial was an attempt to improve on results of CAF chemotherapy in patients with aggressive clinical presentations of MBC using a strategy of multiple drug intensification therapy with a weekly regimen composed of three couplets of drugs.

PATIENTS AND METHODS

Sixty-seven women with MBC were entered on protocol 82 BRE 281 between September 1982 and February 1985. Pretreatment evaluation included history, physical examination, cbc, liver and renal function tests, chest x-ray, liver scan, and bone scan with confirmatory x-rays of bone scan positive areas. Chemotherapy consisted of two doses of CAF given iv at 3-week intervals (C = 500; A = 50; F = 500 mg/m^2). At 6 weeks, antitumor response was assessed using standard accepted criteria (5). Patients not demonstrating tumor progression at 6 weeks were then treated with the following regimen: Days 1-7, oral cyclophosphamide, 150 mg/m^2/day; Day 8, doxorubicin, 35 mg/m^2 iv; Day 15, methotrexate, 200 mg/m^2 iv, followed 1 hour later by 5-fluorouracil, 600 mg/m^2 iv; Day 16, leucovorin, 10 mg/m^2 every 6 hours for six doses orally; Day 22, vincristine, 2 mg/m^2 iv, and prednisone orally, 50 mg/m^2/day on Days 22-29. On Day 29, the entire consolidation regimen was repeated. On Day 58, patients still without evidence of disease progression received CAF × 2 followed by intensification. Patients without progressive disease were then observed for the duration of unmaintained remission. Survival and Duration of Disease Control [(DDC) = months from start of therapy to date off study] curves were calculated with the Kaplan and Meier technique (6). Comparisons of rates were made using chi square statistics. The 95% confidence interval on the induction response rate was lower, 0.17 and upper, 0.39. However, the main objective of this study (rather than response rate) was to increase the median DDC to 16 months. With an alpha error of 0.05, a beta error of 0.10, and an estimated evaluability rate of 85%, we expected to enter approximately 45 patients onto the study. Since the investigational part of the regimen was intensification, additional patients were entered to allow for inevaluability and progression during induction.

RESULTS

A summary of patient characteristics and response appears in table 1. Forty-eight patients had poor-risk (1) presentations including liver, lymphangitic lung, or pleural involvement either with or without soft tissue, bone, or nodular lung metastases. Patients with brain or meningeal metastases were ineligible. Nineteen patients did not have poor-risk presentations as defined above, but each of these patients were felt to have rapidly progressive tumor presentations. Nine had nodular lung metastases with or without bone involvement and ten had locally advanced breast cancer (five inflammatory) with or without other metastatic sites.

Of the 67 patients entered into the trial, the first five

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patients were entered to assess feasibility and tolerability. None developed intolerable toxicity from the intensification regimen leading to expansion to the formal phase II trial. Although all patients entered were eligible for study, eight were considered inevaluable for response to CAF induction (five, insufficient data and three, patient noncompliance). Response to induction for all patients entered was 18 of 67 (27%) [one complete response (CR)]. Despite the relatively low objective response rate, only 11 of 67 (16%) patients progressed or died during induction and a total of 54 patients entered intensification. Eighteen of these 54 improved initial induction response [11 stable disease to partial response (PR) and three to complete response; and four PR to CR]. The final, overall best response for induction plus intensification for all entered patients was CR (12%), CR plus PR (43%), and CR plus PR plus stable disease (72%). Median DDC for all patients was 8.6 months and survival was 11 months. Median DDC for patients stable or better after induction was 8.75 months and survival was 12.7 months.

TABLE 1.—Patient characteristics and response*

Characteristic	No. of patients (%)
Entered	67
Median Karnofsky performance status (range): 80% (35%–100%)	
Median age in yrs (range): 55.5 (24–75.8)	
Estrogen receptors	
No. with positive	15
No. with negative	13
Borderline	6
Unknown	33
Menopausal status	
Premenopausal	(25)
Postmenopausal	(72)
Unknown	(3)
Prior systemic therapy	
Hormones	8
Adjuvant chemotherapy	5
Adjuvant chemotherapy + hormones	5
Dominant metastatic site	
Liver	30 (45)
Lymphangitic lung	9 (13)
Pleural effusion	9 (13)
Nodular lung	9 (13)
Locally advanced	10 (16)
Response	
Best response to entire regimen	29 (43)
Complete response	(12)
Partial response	(31)
Stable disease	(28)
Induction response	
Complete response + partial response	18 (27)
+ stable disease	56 (84)
Median DDC: 8.6 mos	
Median survival: 11 mos	

*100% of the patients were eligible for response.

Among the population as a whole, toxicity to CAF induction was primarily hematologic with associated, non-dose-limiting gastrointestinal toxicity and alopecia. Toxicity secondary to intensification was significant and led to six septic deaths. Three deaths were secondary to patient or physician protocol noncompliance. More patients developed moderate or severe anemia secondary to intensification (21%) as opposed to CAF (6%), although some of this difference could have been related to cumulative red cell depression from the entire regimen. The severity and prevalence of granulocytopenia with CAF (66%) and intensification (51%) were similar, while thrombocytopenia was infrequent with both components of the regimen. Severe neurotoxicity was not a problem, but 34% receiving vincristine developed some degree of peripheral neuropathy. No moderate or severe elevations of serum creatinine were seen due to methotrexate, but 21% of patients developed slight elevations. Congestive heart failure secondary to doxorubicin was not encountered.

DISCUSSION AND SUMMARY

The intensification regimen used after CAF induction in this study included individual drugs known to be active in MBC. Two of the drug couplets (methotrexate and 5-fluorouracil with leucovorin rescue and vincristine plus prednisone) are generally nonmyelotoxic and were used during periods of anticipated myelotoxicity. The arbitrary choice of the cyclophosphamide and doxorubicin sequence at the beginning of intensification was based on sequencing studies in neuroblastoma (7). It was hoped that this overall treatment strategy would increase the 39% response rate and 12% CR rate achieved with CAF alone in poor-risk patients in SECSG 77 BRE 324 (4) and significantly prolong the median DDC of 7.9 months seen in that study. The objective "best" response rate of 43% and CR rate of 12% seen with the current regimen would not appear to be a major advance. Likewise, the DDC of all eligible patients of 8.6 months was not a significant improvement. Even when the DDC analysis is limited to the more favorable subgroup of patients who did not have tumor progression on initial CAF induction, the median DDC of 8.75 months did not warrant the enhanced toxicity of this regimen. While the authors feel that the use of alternating combination therapies given with high dose intensity (8) is a strategy worth exploration in the treatment of MBC, the regimen used in the present trial is too toxic and not effective enough to warrant further study.

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Cisplatin, Doxorubicin, and Cyclophosphamide (PAC) in the Treatment of Mixed Mesodermal Tumor of the Ovary[1,2]

John Wheelock,[3,*] Ken Hancock,[4] and Karl Smith[5]

Sarcoma of the ovary is an uncommon and very aggressive malignancy. These tumors usually present in an advanced stage and are characterized by rapid growth and poor survival (1). Attempts to cure advanced or recurrent sarcoma with irradiation and/or chemotherapy have met with limited success (2,3). Chemotherapy protocols used in the treatment of ovarian sarcomas mimic treatment successes seen in uterine sarcomas; however, the most effective treatment regimen has not been identified. The combination of cisplatin and doxorubicin has shown some efficacy in the treatment of uterine sarcomas (4). This report reviews experience in treating six patients with ovarian sarcoma with cisplatin, doxorubicin, and cyclophosphamide (PAC) or cisplatin and doxorubicin.

Six cases of mixed mesodermal tumors of the ovary were seen. Pathologic reports and histologic slides were available and reviewed by each institution's gynecologic pathologist. The morphologic criteria for mixed mesodermal tumor used in this study are adequately described by Morrow et al (2). The sarcomatous element was described as heterologous in three of the cases in this study and as homologous in the remaining three cases.

The six patients ranged in age from 37 to 73 years (average, 56.5). One patient was nulliparous. Five of the six patients were postmenopausal, and all were Caucasian. No patient had a history of pelvic irradiation or endometriosis. All patients presented with abdominal pain as their chief complaint. Two patients presented with a pelvic/abdominal mass, and three patients were also found to have ascites.

All patients received a total abdominal hysterectomy, bilateral salpingo-oophorectomy, and optimum surgical cytoreduction. Three patients obtained 100% removal of tumor, and no patient had residual tumor > 1 cm in diameter. Among the six patients, one was surgically stage Ia, one patient was stage IIc, three patients were stage III, and one patient was stage IV. No patient received postoperative irradiation.

Adjuvant chemotherapy was started within 4 weeks of surgery and consisted of cisplatin (50 mg/m^2), doxorubicin (50 mg/m^2), and cyclophosphamide (500 mg/m^2) in five patients and cisplatin (50 mg/m^2) plus doxorubicin (50 mg/m^2) in one patient. Each course of treatment was repeated every 4 weeks. The number of chemotherapy courses ranged from two to 11 (median, six). Three patients, all clinically without disease, had a second-look laparotomy. Two of the three patients had a positive second look after six and 11 courses, respectively. Both patients died within 7 months after the second-look surgery. One patient who had a negative second look after eight courses is in clinical remission 12 months after surgery. One patient developed a small bowel obstruction after five courses, and an exploratory laparotomy revealed macroscopic disease. Two other patients were noted to have progression after two and six courses of PAC.

Five of the six study patients died from 3 to 15 months following their initial diagnosis (table 1). As no patient presented at initiation of chemotherapy with measurable disease, no responses (ie, complete or partial) could be determined. Patients with carcinosarcomas survived somewhat longer (9, 15, and 19+ months) than those with heterologous sarcoma elements (3, 11, and 13 months), but the small numbers preclude any analysis.

The mainstay in the treatment of ovarian sarcomas is surgery, but surgery alone will not cure most patients. Definitive therapy would suggest optimal surgical cytoreduction followed by adjuvant chemotherapy and/or

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[2] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Air Force or the Department of Defense.

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TABLE 1.—Patient data*

Patient No.	Age (yrs)	Stage	Initial therapy	Chemotherapy	No. of courses	Second look	Survival (mos)
1	52	III	TAHBSO	PAC	11	Positive	15
				VAC	2		
2	59	III	TAHBSO	PAC	5	N/A	9
				VAC	2		
3	37	IA	TAHBSO	PAC	2	N/A	3
4	54	IV	TAHBSO	PAC	9	N/A	11
				PC	2		
				5FU plus VP-16	2		
5	64	IIC	TAHBSO	PA	8	Negative	19+
6	73	III	TAHBSO	PAC	6	Positive	13
				VAC	5		

*TAHBSO = total abdominal hysterectomy, bilateral salpingo-oophorectomy; PAC = cisplatin, doxorubicin, cyclophosphamide; VAC = vincristine, dactinomycin, cyclophosphamide; 5FU = 5-fluorouracil; VP-16 = etoposide.

irradiation. Chemotherapy protocols used in ovarian sarcomas mimic successes that have been seen in the treatment of uterine sarcomas. Effective chemotherapeutic regimens in uterine sarcomas have been doxorubicin alone or in combination with dacarbazine and the combination of vincristine, dactinomycin, and cyclophosphamide (VAC). Unfortunately, successful remissions are uncommon with these regimens.

Five of the six patients treated in this study died within 15 months despite two early-stage tumors, optimum cytoreduction, and timely initiation of chemotherapy. Survival in these patients is poor and does not justify a larger trial of this regimen. To date, there are no known effective regimens for this disease process.

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BRIEF REPORTS

Iv Melphalan in Children[1,2]

Jean Bello Belasco,[3,*] Christopher D. Mitchell,[4] Thomas Rohrbaugh,[5] and Jeffrey Rosenstock[6]

Forty children with multiply relapsed cancers received iv melphalan at three doses: 30 mg/m², 45 mg/m², and 60 mg/m². The hematologic toxicity was severe and protracted at all dose levels, whether or not the bone marrow was involved with tumor. Of 39 evaluable patients, 37 had grade 3 or 4 hematologic toxicity. Nonhematologic toxic effects were infrequent and not severe. Two complete responses (Hodgkin's disease, rhabdomyosarcoma), eight partial responses, and 30 failures were seen. There appeared to be a very narrow margin between efficacy and toxicity. Further study of melphalan in pediatric tumors may be warranted in special circumstances: in higher doses (> 100 mg/m²) as cytoreduction therapy for specific cancers with marrow rescue, or as part of combination therapy in certain cancers (eg, lymphoma, sarcoma), possibly at doses of 20 to 30 mg/m² every 4 weeks. [Cancer Treat Rep 71:1277-1278, 1987]

There are several reasons for wishing to study iv melphalan in children with cancer. Preliminary reports of children with refractory neuroblastoma and Ewing's sarcoma treated with melphalan, total-body irradiation, and marrow reconstitution have been promising (1-5). Children who have received high-dose cyclophosphamide, especially prior to bone marrow transplantation, have a significant incidence of hemorrhagic cystitis, whereas this complication may be less common with melphalan. There are few data for childhood cancers treated with melphalan by any route of administration. Earlier clinical trials of melphalan in lymphoma, leukemia, and neuroblastoma have used the oral route; however, oral administration is suboptimal because of incomplete absorption (6-9). There are no dose-response data for iv melphalan, although high-dose pulses have been used prior to autologous bone marrow transplantation for neuroblastoma. Data for low doses (up to 1 mg/kg) given iv have been sparse for pediatric tumors, but some responses have been seen in lymphomas and in osteogenic sarcoma.

METHODS

Children eligible for parenteral melphalan therapy had experienced multiple relapses or had become refractory to standard therapy and to other phase II agents. Forty such children were treated at Children's Hospital of Philadelphia between 1980 and 1985. All children eligible for study had measurable disease and a life expectancy of at least 8 weeks. The patients were required to have an absolute neutrophil count (ANC) $\geq$ 1500/mm³ and a platelet count $\geq$ 100,000/mm³ (unless there was bone marrow involvement), creatinine $\leq$ 1.5 mg/dl, and liver enzymes $\leq 2 \times$ normal. Informed consent was obtained prior to treatment, and a child could be removed from study at any time by family request or if unacceptable toxicity or progressive disease occurred.

Following melphalan administration, a cbc with differentials and platelet count were performed weekly. Liver enzymes and BUN and creatinine were checked prior to each administration of melphalan. All patients were assessed for disease response (prior to the second dose of melphalan) at 3-4 weeks.

A single dose of melphalan was given by slow iv push over 5 minutes on Day 0 and then every 3 weeks, depending on bone marrow recovery. Three doses were used: 30 mg/m², 45 mg/m², and 60 mg/m².

A complete response was defined as the disappearance of all identifiable disease, no new disease, and/or conversion of an M3 (> 25% blasts) bone marrow to M1 (< 5% blasts); a partial response was defined as a $\geq$ 50%

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reduction in measurable disease and/or conversion of an M3 marrow to M2 (5%–25% blasts). No response was defined as the lack of complete or partial response and/or persistence of an M3 marrow. The minimum duration of response was defined as 3 weeks. Toxicity assessed was hematologic, hepatic, renal, gastrointestinal, and allergic or anaphylactic.

RESULTS

Forty children were entered on study and prospectively analyzed. With the exception of brain tumors, the tumors treated represented the spectrum of pediatric cancers (table 1). One patient with chronic thrombocytopenia and platelet transfusion dependency secondary to prior therapy for Hodgkin's disease was evaluable for response but not for toxicity. Twenty-two patients received 30 mg/m^2. Five patients received 60 mg/m^2. One of five patients without bone marrow disease died with severe pancytopenia after receiving 60 mg/m^2. Therefore, 13 subsequent patients received 45 mg/m^2.

Hematologic toxicity was severe (generally grade 4) regardless of bone marrow status. Toxicity was seen in all treatment groups but was more severe in patients receiving 45 or 60 mg/m^2. Maximal blood product and antibiotic support were given to all patients. Hemorrhage occurred in 11 of 39 patients despite platelet transfusions for any platelet count $\leq$ 20,000/mm^3; fever and neutropenia requiring antibiotics for presumed sepsis occurred in 17 patients; severe thrombocytopenia ($\leq$ 20,000 cells/mm^3) with no hemorrhage occurred in 11 patients; isolated neutropenia (ANC $<$ 500) occurred in 12 patients. Two patients became refractory to platelet transfusions without evidence of disseminated intravascular coagulation, one of whom had leukemia and had had previous transfusions. This patient in leukemic relapse with documented sepsis died of an intracranial hemorrhage. Three patients died during melphalan therapy. All had grade 4 myelosuppression with overwhelming infection and/or bleeding (one acute lymphoblastic leukemia, one Hodgkin's disease, one osteogenic sarcoma). All three of these patients had progressive disease at the time of death. In the patients with leukemia and with Hodgkin's disease, the florid and extensive nature of their relapsed disease appeared to be the major cause of death. More than 25% of the children evaluated had protracted myelosuppression, ranging from 4–6 weeks following a single dose of melphalan.

When only patients with solid tumors were analyzed, 22 of 23 had grade 4 and one of 23 had grade 3 hematologic toxicity.

Two patients experienced transient elevation of liver enzymes. Two patients had severe but short-lived vomiting. One patient developed the syndrome of inappropriate ADH. No patients developed renal or bladder toxicity or anaphylaxis.

Two complete responses were seen, one each in patients with Hodgkin's disease and rhabdomyosarcoma.

TABLE 1.—Responses to melphalan*

Disease	No. of patients (n = 40)	CR	PR	NR
Leukemia†	15	0	0	15
Non-Hodgkin's lymphoma	4	0	1	3
Hodgkin's lymphoma	3	1	1	1
Neuroblastoma	8	0	4	4
Renal cell carcinoma	1	0	0	1
Wilms' tumor	3	0	2	1
Sarcoma	6	1	0	5

*Values = No. of patients. CR = complete response; PR = partial response; NR = no response.

†13 acute lymphoblastic leukemia, 1 chronic myelogenous leukemia, 1 acute myelogenous leukemia.

The response in the patient with Hodgkin's disease was the longest and lasted 5 months. The patient with rhabdomyosarcoma received a single dose of melphalan with a complete disappearance of tumor mass on chest x-ray. However, severe recurrent hemoptysis prevented the use of further doses of melphalan (table 1). In addition to these complete responses, eight partial responses were obtained in one of four patients with non-Hodgkin's lymphoma, one of three with Hodgkin's disease, four of eight with neuroblastoma, and two of three with Wilms' tumor. The responses seen were of short duration and had no apparent relationship to dose. There were no responses in leukemia (13 of 15 patients had acute lymphoblastic leukemia).

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BRIEF REPORTS

Oral Idarubicin as Single-Agent Treatment of Acute Nonlymphocytic Leukemia in Poor-Risk Patients[1]

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Oral idarubicin was given as single-agent treatment of acute nonlymphocytic leukemia in 18 poor-risk patients. They comprised nine previously untreated elderly patients, age range 69–86, and nine relapsed pretreated patients, age range 41–76. Overall, two patients achieved complete remission (including one with preceding refractory anemia with excess of blasts) and seven achieved partial responses. Dose-limiting toxic effects were diarrhea and sepsis. In this limited study, oral idarubicin at a dose of 20–25 mg/m^2/day × 3 was a well-tolerated drug with potent antileukemic effects. The oral formulation deserves more widespread evaluation. [Cancer Treat Rep 71:1279–1281, 1987]

Idarubicin (IDR) (also known as demethoxydaunorubicin) is a newly developed analogue of daunorubicin. Unlike other drugs of its class, it is effective when administered orally (1). We have investigated its value as a single oral agent to treat acute leukemia in a group of poor-risk patients. The oral regimen was suitable for outpatient use.

MATERIAL AND METHODS

The study design was approved by the Ethics Committee of each institution and patients gave informed consent. The characteristics of the patients and their responses are shown in table 1, where they are divided into two groups—previously untreated elderly patients and relapsed or refractory pretreated patients. The majority of the pretreated patients had received daunorubicin, cytarabine, and, sometimes, etoposide according to the protocol of the Australian Leukaemia Study Group (2). Six of the nine had achieved one or more prior remissions while three had primary refractory leukemia.

Induction treatment was given over 3 consecutive days and consisted of 20 mg/m^2 daily (six patients; range, 1–4 courses; total, 11 courses); 25 mg/m^2 daily (four patients, one or two courses, total, six courses); or 30 mg/m^2 daily (eight patients, one or two courses, total, 10 courses). Thus, patients received induction doses of either 60, 75, or 90 mg/m^2 per course.

The status of the leukemia was re-evaluated 28 days after each treatment by repeat bone marrow (BM) aspiration except in those with persistent peripheral blood blast cells. *Complete remission* (CR) was defined by conventional criteria, namely BM containing < 5% blasts and normalization of peripheral blood values. *Partial response* (PR) was defined as reduction of peripheral blood blast cells to 1%–5% and/or BM with 5%–15% blasts. If CR was not achieved, clinicians had the option of giving further courses. Patients who achieved CR received maintenance treatment at the same daily dosage once each 14 days.

RESULTS AND TOXICITY

Results are given in table 1. Both patients who achieved CR were aged 75 and both had acute nonlymphocytic leukemia (ANLL) of the French, American, British (FAB) M2 variety. One patient, a woman, achieved CR after a single course. The other, a man, had prior refractory anemia with excessive blasts (RAEB); he required two courses to achieve CR. In the former patient, maintenance treatment was ceased after 26 weeks; the CR is still continuing after 70 weeks. The second patient relapsed after 12 weeks and subsequently died. Of the ten patients who received 60 or 75 mg/m^2 induction dose, four achieved PR, whereas of the eight who received 90 mg/m^2 induction dose, two achieved CR and three achieved PR.

[1] Received Apr 7, 1987; revised Aug 25, 1987; accepted Sept 3, 1987.
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TABLE 1.—Patient characteristics and responses*

	Previously untreated elderly patients ($n = 9$)	Pretreated patients ($n = 9$)
Age range (median)	69–86 (75)	41–76 (59)
M:F	7:2	4:5
Peripheral blood blasts ($\times 10^9$/L)	0–200	0–50
Bone marrow blasts (n)	25%–78% (7)	33%–95% (5)
Results		
Complete remission	2	0
Partial response	3	4
Stable disease	0	1
Progressive disease	2	2
Not evaluable due to early (toxic) death	2	2

*Unless otherwise specified, values = No. of patients.

Toxicity details are given in table 2. The drug was well tolerated with minimal nausea and vomiting. However, all patients developed significant hematological toxicity of the type usually associated with aggressive antileukemia treatment. This was often complicated by life-threatening infection, especially in patients who received the highest dose. The other major side effect was diarrhea, which developed in four of the eight (50%) patients who received the highest dose and only in one (an 86-year-old) who received a lower dose. It occurred several days after the drug was administered, lasted for up to 2 weeks, and was bloody in two cases. Only one cardiac event was noted. A 76-year-old man with ANLL supervening on chronic myelomonocytic leukemia (CMML) had a small myocardial infarction during induction treatment. He made a full recovery, his ANLL reverted to CMML, and he remains alive and well, and off treatment, 12 months later.

Maintenance treatment caused mild chronic diarrhea in one patient and no side effects in the other.

TABLE 2.—Relation of dose to incidence of side effects*

	Dose per induction course (mg/m^2)	
	60–75	90
No. of patients	10	8
No. of courses	17	10
Side effect		
Severe diarrhea	1	4 (50%)
Severe infection	3 (30%)	7 (88%)
Nausea and vomiting	1	0
Hepatic dysfunction	2	1
Renal dysfunction	0	1
Drug-related deaths	1	3 (38%)

*Unless otherwise specified, values = No. of patients.

DISCUSSION

In our group of patients, oral IDR proved to be a well tolerated drug with potent antileukemic effects when used in a dose of 60 or 75 mg/m^2. Above this dosage the major side effects were life-threatening infection during neutropenia and severe diarrhea.

Leukopenia developed very rapidly after oral treatment. Although the treatment itself could be administered on an outpatient basis, most patients had to be hospitalized during the period of neutropenia for treatment of infections. We did not routinely administer prophylactic antibiotics such as cotrimoxazole and ketoconazole, but we would recommend their use in future trials in view of the regular development of severe pancytopenia.

When oral IDR was administered at a high dose of 90 mg/m^2, severe and sometimes bloody diarrhea became a major problem. Our experience suggests that gastrointestinal toxicity may be the dose-limiting factor for oral IDR, possibly because the intestinal epithelium is exposed to a higher dose by the oral route. However it is possible that higher doses could be administered safely to a younger and more fit group of patients.

Our study suggests that oral IDR is an effective treatment for acute leukemia. Nearly all patients, including those recorded as having progressive disease or being unevaluable due to early death, had at least a transient antileukemic effect. We believe that the response rate seen in the previously untreated elderly patients was encouraging. It is of particular interest that two patients with prior myelodysplastic syndrome had good responses. One who achieved CR had ANLL developing after prior RAEB, a situation in which CRs are less common than in de novo ANLL (3). Another whose ANLL supervened on CMLL had reversion to CMML. This experience is supported by a recent report that seven of ten patients with RAEB, who were treated with oral IDR alone, had responses (three CRs and four PRs) (4). Oral IDR deserves further evaluation for the

treatment of some myelodysplastic syndromes and for acute leukemia in poor-risk patients when a simple outpatient regimen is desirable.

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BRIEF REPORTS

Phase II Study of Lonidamine in Patients With Small Cell Carcinoma of the Lung[1]

Nevin Murray,* Amil Shah, and Pierre Band[2]

Lonidamine, a substituted indazole carboxylic acid that inhibits cellular respiration, was given in an escalating oral schedule to 20 evaluable patients with measurable extensive small cell carcinoma of the lung. Two partial responses occurred. Reversible acceptable toxicity included myalgia, nausea, hyperesthesia, photophobia, somnolence, and testicular pain. The drug was not myelosuppressive. Lonidamine has modest activity in small cell lung cancer and further studies are warranted. [Cancer Treat Rep 71:1283-1284, 1987]

Lonidamine, an indazole carboxylic acid derivative, interferes with mitochondrial function and inhibits cellular oxygen consumption (1,2). Toxicity in humans includes myalgia, somnolence, nausea, skin hyperesthesia, and testicular pain. Myelosuppression does not occur (3). Lonidamine has shown activity in breast cancer (4) and non-small cell lung cancer (5).

This report summarizes the results of a phase II study of oral lonidamine in patients with small cell lung cancer.

METHODS AND PATIENTS

Twenty-three patients with metastatic small cell lung cancer were entered in the study between April 1983 and May 1985. Eligibility requirements included: histologically confirmed small cell lung cancer, a measurable unirradiated index lesion, Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2, life expectancy of > 8 weeks, no chemotherapy for a minimum of 4 weeks, normal hematologic parameters, bilirubin ≤ 1.5 mg/100 ml, and creatinine ≤ 1.5 mg/100 ml. Written informed consent was obtained from all patients.

Lonidamine was given daily orally in two divided doses using an escalating schedule as shown in table 1. Prednisone (5 mg orally twice daily) was added when moderate or severe myalgia occurred.

Patients were evaluated for response and toxicity from initiation of treatment. Standard criteria of response were used (6).

Of 23 patients entered, 20 were evaluable for toxicity and response. Three patients were inevaluable because they refused to take the medication despite consenting to the trial. Patient characteristics are described in table 2. Most patients previously treated with chemotherapy had multiple regimens as part of alternating chemotherapy research projects (7).

RESULTS

Two of the 20 evaluable patients had a partial response while taking lonidamine at the dose rate of 360 mg/m^2/day. One patient without previous chemotherapy or radiotherapy had a supraclavicular lymph node mass decrease $> 50\%$ for 5 weeks. Another patient with recurrent supraclavicular lymph nodes after treatment with two chemotherapy regimens (cyclophosphamide, doxorubicin, vincristine, and etoposide-cisplatin) and chest irradiation responded for 20 weeks. On progression of biopsy-proven disease on lonidamine, the supraclavicular region was irradiated and the patient is alive and disease free 3 years later without further chemotherapy.

The overall toxicity of lonidamine was mild. There was no myelosuppression or hair loss. Myalgia was the predominant side effect and was found in 85% of patients. Those with troublesome muscle pain were treated with prednisone, 5 mg twice a day, with prompt improvement. Although mild residual myalgia, somnolence (50%), nausea (35%), skin hyperesthesia (35%), and photophobia (10%) were common, tolerance to these side effects developed, and individuals taking this drug > 2 months were usually free of side effects. Four of ten men taking lonidamine experienced testicular pain.

[1] Received Jan 27, 1987; revised Aug 31, 1987; accepted Sept 9, 1987.

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TABLE 1.—Lonidamine dose and schedule

Days	Total daily dose (mg/m^2)
1	60
2	120
3-7	180
8-14	270
15-21	360
22+	450

DISCUSSION

This trial demonstrates modest activity of lonidamine in extensive small cell lung cancer, but the response rate of two of 20 has broad confidence limits (2%–32%). Although a larger trial evaluating more untreated patients could be of interest, we believe the therapeutic potential of this drug is more likely to be realized when it is combined with other modalities.

TABLE 2.—Patient characteristics

Characteristic	No. of patients
Median age in yrs (range): 59 (44-78)	
Sex	
Male	10
Female	10
ECOG performance status	
0	2
1	8
2	10
Prior chemotherapy regimens	
None	4
1	2
2	11
3	3
Prior radiotherapy	14
Measurable disease site	
Primary tumor	5
Supraclavicular nodes	8
Liver	4
Lung metastasis	1
Subcutaneous	2

Lonidamine appears to disrupt energy metabolism without affecting DNA replication. Hahn et al (8) have shown that lonidamine inhibits in vitro recovery from potentially lethal damage caused by x-rays, alkylating agents, bleomycin, and heat. It is possible that lonidamine inhibits repair of sublethal damage induced by conventional chemotherapeutic agents. Since lonidamine has a unique mode of activity different from standard chemotherapeutic agents and is nonmyelosuppressive, further trials of this drug in combination with standard chemotherapy combinations are warranted. A study of lonidamine (300 mg/m^2/day) combined with etoposide and cisplatin for extensive-stage small cell lung cancer is currently in progress.

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BRIEF REPORTS

Cisplatin as a First-Line Treatment in T_2 and T_3 Bladder Carcinoma[1]

H. Havsteen,* H. von der Maase, I. Strøyer, and F. Rasmussen[2]

Twenty-two patients with bladder cancer stage T_2 or $T_3N_xM_0$ received preirradiation treatment with cisplatin. Three complete and seven partial remissions were achieved. Responders received additional cisplatin concomitantly with radiotherapy, for a total of eight complete remissions. Toxicity from cisplatin alone or in combination with radiotherapy was moderate. Seven of the ten responders following preirradiation cisplatin are alive without evidence of disease. All nonresponders have died due to relapse (follow-up time, 46–80 months). Randomized trials with and without preirradiation cisplatin are warranted to establish whether cisplatin improves the prognosis of patients with invasive bladder cancer. [Cancer Treat Rep 71:1285–1287, 1987]

Cisplatin is one of the most widely used cytostatic agents in the treatment of advanced bladder cancer, providing a response rate of about 35% when used as a single agent (1–3). Knowledge of cisplatin as a first-line treatment in bladder cancer is, however, sparse. Thus, the aims of the present study were to evaluate the efficacy of cisplatin as a first-line treatment in patients with T_2 and $T_3N_xM_0$ tumors of the bladder and subsequently to evaluate the feasibility of simultaneous administration of cisplatin and irradiation.

MATERIALS AND METHODS

Patients had to fulfill the following criteria: measurable and previously untreated bladder cancer with histologically proven category T_2 or T_3, without evidence of distant metastases. Twenty-eight patients were entered in the study from 1979 to 1982. Six patients were excluded due to previous cytostatic treatment (two patients), other malignancies (two), or nonevaluable disease (two). Characteristics of the 22 evaluable patients are shown in table 1.

The following examinations were performed initially, after cisplatin, and after irradiation with or without cisplatin: cystoscopy with biopsy from the tumor and bimanual palpation under anesthesia, routine blood samples, x-rays of the lungs, bone scan, Cr-EDTA clearance, and renography or iv urography. Tumor debulking was not attempted.

Cisplatin (50 mg/m^2) was administered with usual hydration on Days 1, 4, 22, 25, 43, and 46 (three courses) on an outpatient basis. In case of complete (CR) or partial (PR) remission, two further courses of cisplatin were given simultaneously with irradiation. Radiotherapy was delivered with an 8-meV linear accelerator to the bladder region on one anterior and two lateral fields, including the small pelvic and the obturator and iliac nodes. Total dose was 40 Gy (2.5 Gy × 16) given in four fractions a week (time dose fraction 71, cumulative radiation effect 1424 reu). All patients gave informed consent to participate in the study which was planned and conducted in accordance with the Helsinki Declaration II.

RESULTS

Primary treatment with cisplatin induced CR in three patients, PR in seven patients, no change in eight patients, and progressive disease in four patients. Thus, the response rate (CR + PR) was 46% (95% confidence interval, 24%–68%). The three patients with CR all had T_2 tumors, and among the patients with PR, three had T_2 and four had T_3 tumors. Among the patients still alive with no evidence of disease (NED), four had T_2 tumors and three had T_3 tumors. Two of the three patients with CR are alive with NED after 74 and 80 months. One of these patients has had a partial resection of the bladder. One patient died from asthma (NED) after 42 months. Among the seven patients with PR,

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TABLE 1.—Patient characteristics*

Characteristic	No. of patients
Evaluable	22
Sex	
Female	6
Male	16
Median age in yrs (range): 57 (38–70)	
Histology	
Transitional cell carcinoma	21†
Tumor stage	
T_2	11
T_3	11
Grade	
II	6
III	11
IV	4
Hydronephrosis	13
Impaired Cr-EDTA clearance	4‡

*According to Bergkvist et al (4).
†2 with squamous cell carcinoma.
‡51, 55, 57, and 62 ml/min.

four achieved CR, one achieved PR, one had no change and one had disease progression after further cisplatin plus 40 Gy. Four patients are alive with NED (46, 55, 66, and 76 months). Two of these patients have had cystectomy due to residual disease or relapse and one had received an additional 20 Gy to the bladder region. Three patients died from rapidly disseminating disease after 4, 10, and 16 months and received only palliative treatment. In the no change/disease progression group (12 patients), further treatment consisted of cystectomy (seven patients), supplementary irradiation to a total dose of 60 Gy (three patients), or palliative treatment because of rapidly disseminating disease (two patients). All patients have died from relapse, with a median survival time of 14.5 months (range, 3–43). In summary, seven of ten patients achieving CR or PR on initial cisplatin treatment are alive with NED except for one patient who died from other reasons. Two of these patients have had a cystectomy performed. In contrast, none of 12 patients with no change or disease progression is alive. Seven of these patients had a cystectomy performed.

The dosage of cisplatin was reduced by 25%–50% in nine patients (four receiving preirradiation and five receiving combined treatment) due to nausea and vomiting (three patients), decreased Cr-EDTA clearance (three), or hearing loss (three). Significant myelosuppression was only seen in one patient with fever: leukopenia (1.0×10^9 cells/L) and thrombocytopenia (32×10^9 cells/L). Median Cr-EDTA clearance before and after cisplatin was 73.5 ml/minute (range, 51–128) and 68 ml/minute (range, 48–116), respectively. One patient developed subileus attacks due to ip metastases and radiation enteritis. Another patient developed abscess and necrosis of the top of the vagina in conjunction with cystectomy.

DISCUSSION

Raghaven et al (5) achieved PR in eight of 12 patients after two courses of cisplatin at a dose of 100 m; previously untreated bladder cancer. Follow-up showed a response rate of 60% among 50 patients, with an actuarial 12-month survival rate of 88% (5). This response rate seems to be higher than that achieved in advanced disease where cytostatic treatment may be hampered by previous antineoplastic treatment, bad general condition, and impaired renal function (6). In the present study, the response rate of 46% is not remarkable but the modest toxicity was surprising.

Two clinical studies with cisplatin combined with irradiation at doses of 45–60 Gy have shown remarkably high CR rates of 76% and 77% with considerable subjective toxicity, whereas the frequency and severity of the long-term complications were unchanged compared with irradiation alone (7,8). Herr et al (9) treated patients with locally advanced bladder cancer with one 70-mg/m^2 dose of cisplatin and radiotherapy at a dose of 20 Gy prior to cystectomy. This combined treatment was widely atoxic and in 21% of the patients no tumor was found in the surgical specimen. Following the combined treatment, we achieved a CR in eight of 22 patients (36%). It is, however, impossible to indicate whether cisplatin has enhanced the irradiation response since the study did not include a control group receiving irradiation alone. In this respect, it should be emphasized that in a randomized study (10) preoperative cisplatin plus radiation therapy was not found to be superior to irradiation alone. However, only one single dose of cisplatin and a radiation dose of 20 Gy were used.

In summary, cisplatin seems to be effective as a first-line treatment in patients with bladder cancer. The drug can be administered either before or simultaneously with irradiation of the bladder region, at least in the present regimen, without causing unacceptable side effects. The efficacy and toxicity of combined irradiation and cisplatin should be finally evaluated by comparison with radiation alone in a randomized study.

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Phase II Evaluation of Orally Administered Idarubicin in Patients With Advanced Breast Cancer[1,2]

Ephraim S. Casper,* Virginia Raymond, Thomas B. Hakes, Violante E. Currie, and Richard J. Kaufman[3]

Doxorubicin is the most active single cytotoxic agent available for the treatment of advanced breast cancer, but chronic cumulative cardiac toxicity has limited its use. In murine models, idarubicin is more potent than doxorubicin and is active against both leukemias and solid tumors. Furthermore, idarubicin is devoid of cardiotoxicity in experimental systems when administered orally (1,2). To evaluate the clinical efficacy and toxicity of idarubicin, we conducted a phase II study of idarubicin in patients with advanced breast cancer who had not been treated previously with an anthracycline.

PATIENTS AND METHODS

Patients with microscopically confirmed breast cancer with clinical evidence of metastatic or locally advanced disease were eligible for this study. Patients may have had prior endocrine therapy and one prior combination chemotherapy regimen, but no previous treatment with an anthracycline. Additional criteria for eligibility included measurable or evaluable disease, Karnofsky performance status of at least 60%, wbc count $\geq 4000/mm^3$ and platelet count $\geq 120{,}000/mm^3$, serum creatinine < 2.0 mg/dl, and serum bilirubin < 1.5 mg/dl. Patients with a history of congestive heart failure or active cardiac disease were excluded from this protocol, as were patients who had received radiation therapy or chemotherapy within 4 weeks.

The pretreatment evaluation included complete history and physical examination, automated blood cell count, biochemical profile, chest x-ray, and radionuclide cineangiography. Additional radiographic and radionuclide scans were performed as needed to define the extent of disease.

The initial dose of idarubicin was 45 mg/m^2 divided into three approximately equal single daily doses given orally on 3 consecutive days. The calculated dose for each patient was rounded off to the nearest multiple of 5 mg. Doses were repeated every 21 days if the wbc count was $\geq 4000/mm^3$ and the platelet count was $\geq 100{,}000/mm^3$. An automated blood cell count, including a platelet count, was obtained weekly. If the wbc count remained $> 4000/mm^3$ and the platelet count remained $> 150{,}000/mm^3$ during a given cycle, the dose in the next course was increased by 5 mg/m^2. If the wbc count nadir was $< 2000/mm^3$ or if the platelet count nadir was $< 75{,}000/mm^3$ for a given course, the next dose was reduced by 10 mg/m^2. Physical examination and biochemical profile were repeated at 3-week intervals.

Indicator lesions were assessed every 3 weeks with the exception of lesions measured by radionuclide or computerized tomographic scans, which were repeated at 6-week intervals. Standard response criteria were employed. Radionuclide cineangiography was repeated after a cumulative dose of 200 mg/m^2 and after every additional 80 mg/m^2 thereafter.

RESULTS

The characteristics of the 22 women who were entered in this study are shown in table 1. No patient achieved complete or partial therapeutic response; however, one patient had unequivocal improvement of an evaluable chest wall lesion lasting 8 months. Two other patients experienced minor regressions of measurable disease, each lasting 3 months. Five patients had stable disease for 3, 3, 8, 8, and 9 months.

The median number of doses administered was 2.5 (range, one to 11 doses). One patient was excluded from the toxicity analysis because she received three times the protocol dose. During treatment, the dose of idarubicin was escalated in seven patients and was reduced in six on the basis of hematologic effects. One patient

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Entered	22
Evaluable	22
Median age in yrs (range): 54 (30–83)	
Median performance status (range): 80% (60%–90%)	
Prior chemotherapy	
None	6
Adjuvant only	8
Adjuvant and palliative	2
Palliative only	6
Dominant site of disease	
Soft tissue/nodes	13
Lung/pleura	4
Bone	3
Viscera	2
Advanced primary lesion	0
Hormone receptor status	
Estrogen receptor +	5
Estrogen receptor −	8
Estrogen receptor unknown	9
Median wbc count nadir, × 1000/mm^3 (range): 2.3 (0.4–11.1)	
Median platelet count nadir, × 1000/mm^3 (range): 158 (40–389)	

died of staphylococcal sepsis associated with neutropenia 7 days after receiving her fourth dose of idarubicin; her wbc count was normal on the day of idarubicin administration. This patient had achieved a minor regression in pulmonary nodules but developed brain metastases and received whole-brain radiotherapy during treatment with idarubicin.

Sixteen patients (76%) had nausea, including nine who also had vomiting. Eight patients (38%) had diarrhea which was mild to moderate. Some hair loss was seen in 50% of the patients, but only five lost 50% of their hair and none had total alopecia. Three patients reported mucositis. There were no episodes of clinical congestive heart failure, and among the five patients who had repeat cineangiography, no abnormalities in ejection fraction were detected after 196, 206, 286, 340, and 508 mg/m^2 of idarubicin.

DISCUSSION

No complete or partial responses were achieved in our trial. Other investigators have reported responses in 23%–50% of patients treated with oral idarubicin (3–8). There is no obvious explanation for this discrepancy. Eight patients in this study did exhibit evidence of therapeutic effect (one had disease improvement, two had minor regressions, and five had stable disease); however, none met the criteria for partial response. Although 16 of our patients had received prior chemotherapy, they were of good performance status and received myelosuppressive doses of idarubicin. Most patients had soft tissue disease. In a previous Memorial Hospital study conducted with a similar patient population and using the same response criteria, doxorubicin and epirubicin each produced major objective responses in 25% of patients (9). We conclude, therefore, that in this dose and schedule, idarubicin has minimal activity in patients with advanced breast cancer.

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Phase II Trial of Mitoxantrone as First-Line Chemotherapy for Extensive Small Cell Lung Cancer[1]

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Anthracycline antibiotics, notably doxorubicin, have substantial single-agent activity in small cell lung cancer (1). Mitoxantrone has been shown to give response rates similar to those of doxorubicin in advanced breast cancer and is associated with less toxicity (2). A phase II trial of mitoxantrone in previously untreated patients with small cell lung cancer is reported. The characteristics of the patients studied are shown in table 1.

Mitoxantrone was given as an infusion in 250 ml of 5% dextrose over 30 minutes. A dose of 14 mg/m^2 was administered on the basis of the doses used in breast cancer (2). Two patients received 12 mg/m^2 due to preexisting neutropenia. Chemotherapy was given every 3 weeks, and patients were assessed for response in evaluable sites at the end of each treatment cycle. A total of 15 patients were studied. Criteria for eligibility in the study included: (*a*) documented extensive small cell lung cancer; (*b*) measurable disease; (*c*) normal liver and renal functions; (*d*) no previous history of congestive cardiac failure (resting ejection fraction $> 50\%$); and (*e*) no previous radiotherapy or chemotherapy.

RESULTS

None of the patients entered in the study had objective response to mitoxantrone. Two patients died after the first course of treatment due to the malignant process on Days 8 and 18, respectively. The mean wbc count nadir for the total number of treatment cycles was 2.16×10^9/L, and the mean platelet count nadir was 156×10^9/L. A wbc count $< 1 \times 10^9$/L was documented in only one patient. One patient experienced severe nausea and vomiting. None of the patients developed alopecia or hepatic or renal toxicity. Cardiotoxicity was assessed by serial electrocardiographs and measurement of ejection fraction every three cycles; there was no cardiotoxicity in any patient. Three of the nonresponding patients were given further chemotherapy. Two patients were treated with combination vincristine (1.4 mg/m^2), doxorubicin (50 mg/m^2), and cyclophosphamide (500 mg/m^2), and no response was noted. A third patient received vincristine (2 mg) and etoposide (200 mg/m^2/day orally for 5 days) and also did not respond. Palliative mediastinal radiotherapy was given to two additional patients, who had partial response. The median survival of the patients in the study after commencing treatment was 8 weeks (range, 8 days to 30 wks).

DISCUSSION

For assessment of the usefulness of a cytotoxic drug in the treatment of cancer, it is important to define its activity in untreated patients, as previous chemotherapy and radiotherapy can lead to chemoresistant disease.

TABLE 1.—Patient characteristics*

Characteristic	
Entered	15
Age (yrs)	
Median	65
Range	49–76
Sex	
Male	12
Female	3
Karnofsky performance status	
Median	70
Range	70–100
No. of cycles of mitroxantrone	
Median	2
Range	1-5
Response	
Complete	0
Partial	0
Disease progression	15

*Unless otherwise specified, values = No. of patients.

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This study shows that even when mitoxantrone was used as first-line treatment for small cell lung cancer, it was ineffective in the treatment of this disease. A similar result has been noted in previously treated patients with small cell lung cancer (3). This is in contrast to the results reported in breast cancer, in which mitoxantrone has activity comparable to that of doxorubicin (2). These results highlight the differences in the chemosensitivity of different tumors to structurally similar cytotoxic agents.

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Phase II Study on Oral *N*-Methylformamide in Metastatic Colorectal Cancer[1,2]

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N-Methylformamide (NMF) is a polar planar solvent. The precise mechanism of action is unclear, but it appears that the drug induces cellular differentiation and causes depletion of cellular reduced glutathione, resulting in inhibition of cell growth (1). The NMF showed activity against CX_1 colon tumors in the National Cancer Institute human xenograft panel. In phase I studies, the toxic effects were mainly gastrointestinal, and hepatotoxicity was the dose-limiting factor (2-4). Because the drug is well absorbed after oral administration, we performed a phase II study with oral NMF in advanced, measurable colorectal cancer.

METHODS

The NMF was given at a dosage of 400 mg/m^2 orally twice daily for 5 days every 3 weeks. Dose reduction of 50% was made only if hyperbilirubinemia developed. Patients were required to have an Eastern Cooperative Oncology Group performance status of 2; age < 70 years; leukocyte count > 3000/mm^3; platelet count $> 100{,}000$/mm^3; and serum bilirubin < 2.0 mg/dl.

All patients had bidimensionally measurable disease with cytological proof of metastases. Fourteen patients were pretreated with 5-fluorouracil or esorubicin (4'-deoxydoxorubicin). Response to treatment was estimated after every second cycle according to the World Health Organization criteria (5).

RESULTS

Twenty-six patients were entered in the study (table 1); 22 were fully evaluable for response. We observed one partial response of 7 months' duration in a pretreated patient with liver metastases; six patients had stable disease, with a median time until disease progression of 12 weeks (range, 9-27). Fifteen patients had early disease progression.

Twenty-five patients were evaluable for toxicity. All patients had nausea and vomiting (11, grade 3; one, grade 4). Other toxic effects were upper abdominal pain (six patients) and retrosternal pain (three) requiring analgesic agents, malaise (three), and feelings of drowsiness (three). Because of the toxic effects, two patients refused further treatment after the first course and one after the second. Deterioration in liver function was observed only in patients with progressive liver metastases.

TABLE 1.—Patient characteristics*

Characteristic	
Total No. of patients	26
Male:female ratio	15:11
Median age in yrs (range)	59 (37-69)
Median performance status (range)	1 (0-2)
Evaluable for response	22
Refusal after 1st course	2
Grade IV toxicity	1
Lost to follow-up	1
Evaluable for toxicity	25
Total No. of courses	64
Median No. of courses (range)	2 (1-7)
Location of metastases	
Liver only	13
Lung only	5
Liver + lung	3
Liver + soft tissue	5

*Unless otherwise specified, values = No. of patients.

DISCUSSION

In this phase II study, we treated 26 patients who had metastatic colorectal cancer with oral NMF. Although we observed one partial response in a pretreated patient, we conclude that this drug is minimally active in this schedule and has considerable toxicity.

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[2] Supported in part by The Netherlands Cancer Foundation.
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Phase II Trial of Dichloromethotrexate in Patients With Advanced Squamous Cell Carcinoma of the Cervix: A Gynecologic Oncology Group Study[1,2]

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Chemotherapy for advanced squamous cell carcinoma of the cervix has been unsuccessful due to the lack of an active chemotherapeutic agent. In an attempt to fill this void, the Gynecologic Oncology Group (GOG) began a protocol to test a series of potentially active drugs. Methotrexate is known to have limited activity against cervical cancer (1). However, in vitro studies have suggested that compared to methotrexate, dichloromethotrexate appears to have superior activity (2,3). In addition, preliminary data demonstrated a high response rate (76%) when the combination of cisplatin and dichloromethotrexate was used against cervical cancer (4). When cisplatin was used as a single agent, a lower response rate (23%) was achieved (1). Thus, a phase II study was activated using dichloromethotrexate alone against cervical cancer.

Patients with histologically confirmed, measurable squamous cell carcinoma of the cervix were eligible. None of them were candidates for any other form of therapy. Forty patients were entered in the study from January 1984 to October 1985; 36 were eligible and evaluable.

Dichloromethotrexate was given at a dose of 550 mg/m^2 iv every 7 days. This had been determined to be the maximal tolerated dose in a phase II trial (4). In patients with serum albumin < 3.5 g/dl, the dose was reduced to 350 mg/m^2. This dose reduction was necessary to safely treat nine patients.

When the standard GOG definitions (5) for response were applied to the evaluable patients, there were none with complete response; three (8.3%) with partial response; 17 (47.2%) with stable disease; and 17 (47.2%) with disease progression. Patient characteristics are shown in table 1. Age, performance status, tumor grade, dosage regimen, and prior therapy appeared to have no effect on response.

When dichloromethotrexate was given at 550 mg/m^2, there was limited toxicity. Bone marrow toxicity was seen in 13 patients. One developed thrombocytopenia

TABLE 1.—Patient characteristics*

Characteristic	
Age in yrs	
Median	48
Range	26–80
Grade	
1	3
2	22
3	12
Prior radiotherapy	36
GOG performance status	
0	14
1	13
2	10
No. of courses	
Median	9
Range	3–29

*Unless otherwise specified, values = No. of patients.

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[2] Supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in the study: University of Rochester Medical Center, NY (CA-12482); University of Mississippi Medical Center, Jackson (CA-13633); University of Miami School of Medicine, FL (CA-37234); Indiana University Medical Center, Indianapolis (CA-21720); Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC (CA-21946); and The Albany Medical College, Union University, NY (unfunded). J. A. Blessing is a Cancer Research Scientist V.

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with a nadir count of 42,000/mm^3; the remaining 12 had leukopenia with a median wbc count nadir of 3050/mm^3 (range, 1800–3600). The other common sites for toxicity were in the gastrointestinal system; 21 patients experienced mild to moderate nausea and vomiting.

Other adverse effects were self-limited and generally low grade: anemia (eight patients), azotemia (eight), malaise (two), stomatitis (two), elevated liver enzymes (one), headache (one), and cutaneous rash (one).

The activity of dichloromethotrexate used as a single agent for squamous cell carcinoma of the cervix seems to be low; only 8.3% of the patients had an objective response. Nevertheless, preliminary studies show the activity of the combination of dichloromethotrexate and cisplatin to be 76% (4). This is higher than the response rate of 23% for cisplatin alone.

When delivered at a dose of 550 mg/m^2, this drug has limited toxicity. All toxic effects were minor and self-limited, except for grade 3 anemia in three women. Thus, this drug is well-tolerated but has no significant activity as a single agent at the dose and schedule administered in this study for advanced squamous cell carcinoma of the cervix.

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Phase II Study of Tiazofurin in Colorectal Cancer: A National Cancer Institute of Canada Study[1]

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Tiazofurin is a synthetic C-nucleoside that has shown activity in a variety of experimental tumor models. It exerts its effect by inhibiting inosine monophosphate dehydrogenase (1). Toxic effects in phase I studies included myelosuppression, peeling of the skin, skin rash, muscle pain, elevated creatine phosphokinase (CPK), headaches, and neurotoxicity. These toxic effects were dose and schedule related but often occurred in an unpredictable manner (2-5). This study was initiated to determine the activity of tiazofurin in previously untreated patients with colorectal cancer.

MATERIALS AND METHODS

Criteria for patient eligibility included measurable metastatic colorectal cancer; informed consent; Eastern Cooperative Oncology Group performance status ≤ 2; normal bone marrow; and normal renal and hepatic functions. Tiazofurin was administered by rapid iv infusion at a starting dosage of 1650 mg/m^2 daily for 5 days every 21 days. On the day prior to treatment, allopurinol was administered at 300 mg/day orally for 7 days. Complete response was defined as disappearance of all evidence of tumor for a minimum of 3 weeks. Partial response was defined as a $\geq 50\%$ decrease in the sum of the products of the diameters of lesions for a minimum of 3 weeks with no appearance of new lesions. Progressive disease was defined as a $\geq 25\%$ increase in the size of any lesion or the appearance of new lesions. Stable disease was defined as a $< 25\%$ increase in tumor volume or a $< 50\%$ decrease in tumor volume for at least 6 weeks.

RESULTS

Twenty-one patients were entered in the study. One patient was ineligible because the tumor was not colorectal in origin. Three patients were inevaluable for response; one had early death that was not drug related and two withdrew from the study early due to drug toxicity. Patient characteristics and responses are shown in table 1. Hematologic toxicity was mild and not dose related. Nonhematologic toxic effects were common and troublesome. Their incidence and degree of severity are shown in table 2. Muscle pain and weakness occurred in 50% of the patients; CPK elevation did not correlate with their incidence or severity. The arrhythmias encountered consisted of sinus bradycardia and atrial fibrillation; they did not produce any symptoms. The most serious toxic effects were neurological; severe

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total eligible/evaluable for toxicity	20
Evaluable for response	17
Sex	
Male	10
Female	10
Median age in yrs (range): 64 (34-78)	
Site of metastases	
Liver	15
Lung	7
Total No. of cycles: 52	
Median No. of cycles/patient (range): 3 (1-4)	
Response	
Complete	1*
Partial	0
Stable disease	6†
Disease progression	10

*6 mos.
†Median, 2 mos.

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TABLE 2.—Nonhematologic toxic effects*

Toxic effect	No. of patients			
	Mild	Moderate	Severe	Total
Nausea/vomiting	7	4		11
Anorexia	5	1	1	7
Stomatitis	1	3		4
Diarrhea	2			2
Muscle pain and weakness	3	6	3	12
Increased CPK		10		10
Seizure			3	3
Confusion	2	1		3
Dizziness/ataxia	2	1	1	4
Restlessness	3			3
Headaches	7	3		10
Pericarditis/pleuritis	1	1		2
Arrhythmias		2		2
Peeling skin	4	5		9
Skin rash	2	2		4

*$N = 20$ patients. Worst degree of toxicity by patient.

headaches occurred in ten (50%) of the patients and seizures in three (15%). These nonhematologic toxic effects did not correlate with drug dosage and were troublesome in many cases, necessitating discontinuation of treatment in three patients and amendment of the protocol by lowering of the starting dose. The observation of one response in 17 patients excludes a true response rate > 25% with 95% confidence limits. In summary, the use of tiazofurin in this schedule was associated with significant toxicity and did not show any significant activity in colorectal cancer.

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Phase I Trial of *N*-Methylformamide in Pediatric Patients With Refractory Leukemias[1,2,3]

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N-Methylformamide (NMF) is a small polar-planar compound, one of a novel class of antineoplastic agents which has antitumor activity in preclinical models and has been shown to induce differentiation in vitro of the Friend erythroleukemia and HL-60 promyelocytic cell lines (1-3). Based on these preclinical characteristics, NMF was selected for a phase I trial in pediatric patients with leukemias resistant to conventional therapies.

Fifteen patients with refractory acute leukemia were treated (nine lymphoid and six myeloid). There were 12 males and three females, aged from 9 months to 18 years (median, 8 years). All had had intensive prior chemotherapy (median number of previous antileukemia drugs received, 10; range, 5–14).

NMF was administered iv daily × 5 in 200 ml of normal saline over 15 minutes. Patients who tolerated a first course received a second course 10–14 days later. The initial dosage investigated was 800 mg/m^2/day. Subsequent dosages investigated were 900 mg, 1000 mg, and 1100 mg/m^2/day. No intrapatient dosage escalation was performed. Toxic effects and response were assessed by standard criteria in use by clinical cooperative groups (Pediatric Oncology Group).

Twenty-three courses of NMF were administered to 15 patients. Six patients were able to receive a second course, and one a third course, but four of the 23 courses had to be interrupted after two, three, or four doses due to disabling side effects. The toxicity observed is outlined in table 1 according to dosage level. Anorexia, malaise, and moderate to severe nausea and vomiting were the most common side effects of NMF treatment, occurring in 70% of courses overall, but in ten of 11 courses (90%) at ≥ 1000 mg/m^2, in some instances requiring hospitalization for iv hydration and antiemetics. Reversible hepatic toxicity evidenced by elevation of bilirubin and/or transaminases occurred in six of 23 courses (26%). The peak bilirubin observed was 3.8 mg/dl, and the peak transaminase elevation was > 1000 U/L.

TABLE 1.—Toxic effects of NMF

Iv dosage (mg/m^2/day × 5)	No. of patients/ No. of courses	No. of toxic patients*	No. of courses associated with—			
			Nausea/ vomiting	Hyperbilirubinemia	Hepatic transminase elevation	Chest pain and/or abdominal pain
800	4/7	1	4	0	2	3
900	3/5	1	2	0	0	1
1000	6/9	5	9	0	3	3
1100	2/2	1	1	1	1	0
	15/23	8	16 (70%)	1 (4%)	6 (26%)	7 (30%)

*Patients experiencing grade 3 or grade 4 or subjectively intolerable degrees of toxicity.

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[3] *N*-Methylformamide was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

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By far the most clinically significant and unacceptable toxic effect observed was a remarkable syndrome of chest pain. Five episodes of chest pain occurred in four patients and four of these five episodes were excruciating, requiring narcotics for relief, and often being associated with other symptoms, such as a burning sensation, profuse sweating, numbness or pain in both arms, dizziness, headache, and abdominal pain. Ettinger et al (5) describe one patient among 35 adults treated with NMF who experienced two episodes of severe chest pain, associated with dyspnea, malaise, nausea, and vomiting, each episode following a weekly dose of 2000 mg/m^2.

We conclude that the maximum tolerated dose (MTD) of NMF in children and adolescents is 900 mg/m^2/day iv $\times$ 5, similar to the 800 mg/m^2 recommended by McVie et al (4). Though 11 of 15 patients were treated at the MTD or above, no antileukemic effects were noted in any patient.

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Phase II Trial of Combined 5-Fluorouracil Plus Doxorubicin Plus Cisplatin (FAP Regimen) in Advanced Gastric Carcinoma[1]

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The chemotherapy of advanced gastric cancer has been marginally effective in survival prolongation, with response rates of 20%–45% (1–5). It has revolved until recently around combinations of the most active phase II agents, 5-fluorouracil (5-FU), doxorubicin, mitomycin, and cisplatin, all of which have a ≈20% response rate. We report here on the combination of 5-FU, doxorubicin, and cisplatin (FAP), which, given every 4 weeks, is as effective as and better tolerated than 3-week-interval regimens.

MATERIALS AND METHODS

Thirty-two patients seen at the Institut Gustave-Roussy between March 1983 and March 1986 with the diagnosis of advanced gastric carcinoma, confirmed histologically, and previously untreated with chemotherapy were given 5-FU (400 mg/m^2/day × 3 [1-hour iv perfusion]), doxorubicin (50 mg/m^2 iv push on Day 2), and cisplatin (90 mg/m^2 with hydration and mannitol, also on Day 2). Courses were repeated every 4 weeks. Standard baseline biochemical parameters for cisplatin and doxorubicin were determined, metoclopramide-based antiemesis was administered, and European Organization for Research and Treatment of Cancer (EORTC) standard dose reduction schemes were followed. Evaluation was done following World Health Organization (WHO) criteria. Patient characteristics are shown in table 1, as well as a summary of extent of disease, treatment, toxicity, and responses.

RESULTS

All 32 patients were evaluable for activity and toxicity. Complete responses were seen in three patients (9.4%) for 7, 8, and 9 months' response duration. Two of them were surgically confirmed. Partial responses were seen in eight patients (25%) for a median duration of 4 months and a median survival of 8 months. Ten patients were considered to have stable disease, with a

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Entered	32 (100)
Evaluable	32 (100)
Male/female	17/15 (53/47)
Performance status (WHO)	
0	5 (15.5)
1	11 (34.5)
2	14 (43.7)
3	2 (6.3)
Histology	
Well or mildly differentiated	15 (47)
Undifferentiated	3 (10)
Linnitis	14 (43)
Prior treatment or surgery	15 (47)
No. of cycles (n = 139)	
1	2
>6	11
Average: 4.3	
Toxicity (WHO criteria)	
Hematological (no. of cycles)	
Wbc count	
Grade 1: 1	
Grade 2: 1	
Grade 4: 1	
Rbc count	
Grade 1: 5	
Grade 2: 2	
Platelet count	
Grade 1: 1	
Grade 2: 1	
Grade 4: 1	
Digestive	25 (78)
Nausea/vomiting	
Grade 1	4
Grade 2	15
Grade 3	4
Grade 4	1
Mucositis	
Grade 2	1
Alopecia	
Grade 2	28 (90)

continued

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TABLE 1—*continued*

Characteristic	No. of patients (%)
Response*	
CR	3 (9.4)
PR	8 (25)
CR + PR	11 (34)
Stable disease	10 (32)
Progressive disease	11 (33.6)

*CR = complete response; PR = partial response.

duration and survival similar to those of the partial responders (3.5 and 7.5 months, respectively). Objective responses (complete + partial) were 11 of 32 (34.4%; confidence limits, 20%-50%).

If performance status is taken into consideration, the response rate was better for patients in good condition (four objective responses among five patients with World Health Organization [WHO] grade 0); however, five of 14 patients with grade 2 performance status had objective responses. Neither of the two patients with grade 3 WHO responded. There was a 15% 1-year survival, with an overall median survival of 6 months. Toxicity was graded following WHO guidelines. One toxic death occurred when dose reduction guidelines for grade 3 toxicity were not respected after the third cycle. The patient had a bleeding episode and a platelet count $< 4000/mm^3$. The rest of the toxicity and tolerance characteristics are shown in table 1.

DISCUSSION

Our results are in general agreement with the relative efficacy of the FAP regimen reported in gastric carcinoma, with a response rate of 34.4%. We confirm the previous results reported as an abstract on a smaller series of patients (7). We have not observed important toxicity, as other authors have reported (6), probably because of the interval in our scheme and the dose reduction schedule in case of toxicity.

The FAP regimen seems to be a moderately effective combination for the treatment of gastric carcinoma and needs to be compared with 5-FU alone as well as with the other reputedly active regimens.

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Phase II Trial of Etoposide in the Management of Advanced and Recurrent Leiomyosarcoma of the Uterus: A Gynecologic Oncology Group Study[1,2]

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In 1977, members of the Gynecologic Oncology Group (GOG) initiated a series of phase II trials designed to test new agents in a number of different advanced or recurrent gynecologic malignancies, including leiomyosarcoma of the uterus.

Etoposide was chosen for the current trial because of its activity in several nongynecologic tumors (1) as well as modest activity against ovarian adenocarcinoma (2). It was administered at a dose of 100 mg/m^2 diluted in 150 ml of normal saline over a 45-minute period on Days 1, 3, and 5. The cycle was repeated every 28 days. A complete description of materials and methods is contained in an earlier paper (2).

Complete response was defined as the disappearance of all gross evidence of disease for at least 4 weeks. Partial response was defined as a $\geq$ 50% reduction in the product of the largest diameter and its perpendicular of each measurable lesion for at least 4 weeks. Increasing disease was defined as a $\geq$ 50% increase in the product of the largest diameter and its perpendicular of any lesion documented within 8 weeks of study entry or the appearance of any new lesion within 8 weeks of entry into study. Stable disease was defined as any disease not meeting any of the above criteria.

Between July 1978 and November 1985, 28 evaluable patients were accrued. Patient characteristics are summarized in table 1. One complete response (lung nodules initially measuring 1.5 × 1.3 cm) lasting 2.5 months and two partial responses (pelvic and abdominal mass) lasting 2 and 4.5 months were noted; ten patients had stable disease, while 15 had increasing disease. The observed response rate was 10.7% (95% confidence intervals, 3%–26%). Two of the responders to etoposide had previously received chemotherapy (doxorubicin and cisplatin).

TABLE 1.—Patient characteristics*

Characteristic	
Total evaluated	28
Age in yrs	
Median	58
Range	17–75
GOG performance status	
0	10
1	14
2	4
Site of disease	
Pelvis	5
Outside pelvis	23
Prior surgery	
Total abdominal hysterectomy/bilateral salpingo-oophorectomy	23
Laparotomy and biopsy	3
Simple hysterectomy	1
None	1
Prior radiotherapy	7
Prior chemotherapy	27
No. of courses	
Median	2.0
Range	1–8

*Unless otherwise stated, values = No. of patients.

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[2] Supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in the study: University of Alabama, Birmingham (CA-12484); Duke University Medical Center, Durham, NC (CA-12534); University of Rochester Medical Center, NY (CA-12482); Illinois Cancer Council, Chicago (CA-27806); Colorado Foundation for Medical Care, Denver (CA-15975); Georgetown University Hospital, Washington, DC (CA-16938); University of North Carolina School of Medicine, Chapel Hill (CA-23073); University of Texas Health Science Center, Dallas (CA-28160); Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC (CA-21946); University of California Medical Center, Irvine (CA-23765); Stanford University Medical Center, Stanford, CA; Jacksonville University Hospital, FL; University of Kentucky, Louisville; University of New Mexico, Albuquerque; and Mary Imogene Bassett Hospital Community Clinical Oncology Program, Boston, MA (CA-35132).
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Neither had responded. None of the responders had received prior radiotherapy.

Leukopenia was the most frequently encountered adverse effect and was observed in 13 of the 28 patients, with a median wbc count nadir of 3200/mm^3 (range, 1100–3900). Mild to moderate alopecia was seen in five patients, and moderate nausea and vomiting was noted in five patients. Hypotension or other allergic reactions were not observed.

At the dose and schedule tested, etoposide appears to have moderate activity in the treatment of advanced or recurrent leiomyosarcoma of the uterus. Further trials in patients who have received no prior chemotherapy are clearly indicated.

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Phase II Study of Spirogermanium in Advanced Adenocarcinoma of the Prostate: A Southwest Oncology Group Study[1,2]

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Spirogermanium is an experimental nonmyelosuppressive chemotherapeutic agent with activity in a number of animal systems (1,2). Because of the lack of marrow toxicity, the Southwest Oncology Group chose to evaluate this agent in a phase II study in advanced, hormonally resistant adenocarcinoma of the prostate.

PATIENTS AND METHODS

Eligibility requirements included histologically proven advanced adenocarcinoma of the prostate with measurable or evaluable disease, no more than one prior chemotherapy regimen, life expectancy of 6 weeks, performance status of 2 or better (Southwest Oncology Group criteria), serum creatinine < 2.0 mg/dl, and bilirubin < 2.0 mg/dl. No concomitant radiation therapy was allowed.

The initial dose of spirogermanium was 100 mg/m^2 three times per week iv over 45 minutes, with escalation by 10 mg/m^2 weekly to a maximum of 120 mg/m^2 if toxicity did not occur. This was the dose recommended for phase II studies, with neurologic side effects being dose-limiting (3).

The response criteria used were those advocated by the National Prostate Cancer Project (4).

RESULTS AND DISCUSSION

Forty-three patients were registered, and 42 were eligible. The one ineligible patient had received more than one prior chemotherapy regimen. Patient characteristics are given in table 1. There were five early deaths, three due to tumor and one each from pulmonary embolism and myocardial infarction. Three patients refused further therapy. Of the 42 eligible patients, one (2%) had a partial response and eight (19%) had stable disease. The partial response lasted 13 weeks, and stable disease lasted from 9–29 weeks, with a median of 14 weeks.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Eligible	42
Median age in yrs (range): 66 (48–83)	
Median performance status (range): 2 (0–2)	
Prior treatment	
Hormonal therapy only	12
Radiation + hormonal therapy	10
Chemotherapy + hormonal therapy	11
Chemotherapy + radiation + hormonal therapy	9
Disease extent	
Bone only	28
Measurable	14
Toxic effects	
Nausea, vomiting, anorexia	21
Neurologic (not specified)	13
Dizziness	11
Somnolence	8
Phlebitis	3
Leukopenia	3
Stomatitis	2

Toxicity was acceptable. There were no life-threatening or fatal toxic effects. Severe toxic effects which occurred included two patients with nausea/vomiting and anorexia and one each with ataxia and confusion, weakness, disorientation, paresthesias, and drowsiness.

This study shows that spirogermanium, when given by this schedule, is well tolerated but not active in advanced, hormonally resistant adenocarcinoma of the prostate.

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Teniposide in Metastatic Renal and Bladder Cancer: A Southwest Oncology Group Study[1,2]

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Teniposide (VM-26) is a semisynthetic podophyllotoxin derivative. This multi-institutional study was initiated to determine the drug's efficacy in renal cell carcinoma and transitional cell carcinoma of the bladder.

METHODS

Eligible patients with measurable advanced renal cell carcinoma or transitional cell carcinoma of the urothelial system were placed on weekly VM-26 therapy. Patients were required to have a wbc count $> 4000/mm^3$ and platelet count $> 100,000/mm^3$. Adequate liver and renal functions were necessary for entry. Prior radiation and chemotherapy were allowed if 4 weeks had elapsed. All patients who initiated therapy were evaluated for response and toxicity. VM-26 was administered at 100 mg/m^2 iv as a 45-minute infusion weekly $\times$ 4. The 4-week course of VM-26 was repeated after 2 weeks' rest with a wbc count $> 3000/mm^3$ (granulocyte count $> 1500/mm^3$) and platelet count $> 100,000/mm^3$. Dose adjustment for subsequent courses was allowable depending on nadir counts during the four weekly doses.

RESULTS

Patient Characteristics

Eighty-four patients were registered from 28 Southwest Oncology Group (SWOG) institutions (table 1). Two patients were judged ineligible. Of the 82 eligible patients, 23 patients had transitional cell carcinoma of the bladder and 59 patients had renal cell carcinoma. In both groups there were at least twice as many male patients as female patients. There was only one patient with transitional cell carcinoma who had not been previously treated, whereas 16 (27%) renal cell carcinoma patients were previously untreated. Toxicity and response were determined according to SWOG criteria.

Response and Survival

Renal cell carcinoma.—Eight of the 59 patients were not evaluable for response but have been included in the denominator of rate calculations. There were no complete responses and one partial response, which lasted for 6 months before progression. Stable disease was noted in 23 of 57 (40%) patients. The overall median survival was 6.5 months. The 16 previously untreated patients had a median survival of 4.7 months. There were no significant differences in survival between patients previously untreated or treated with chemotherapy alone, radiotherapy alone, or both (log rank test).

Transitional cell carcinoma of the bladder.—Eight of the 23 patients were not evaluable for response but have been included in the denominator of rate calculations. There were no complete responses and one partial response, which lasted for 5 months before progression. Stable disease was observed in 2 of 23 (8%) patients. The median survival was 2.8 months. Only one patient was previously untreated. There were no significant differences in survival between patients treated with chemotherapy alone, radiotherapy alone, or both (log rank test).

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TABLE 1.—Patient characteristics and toxicity*

	Transitional cell	Renal cell
Eligible	23	59
Previous treatment		
None	1 (4)	16 (27)
Chemotherapy only	5 (17)	18 (31)
Radiotherapy only	4 (22)	6 (10)
Chemotherapy + radiotherapy	13 (57)	19 (32)
Sex		
Male	16 (70)	46 (78)
Female	7 (30)	13 (22)
Toxic effect		
Leukopenia	9 (39) (1 fatal)	9 (15) (1 fatal)
Anemia	2 (9)	5 (8)
Thrombocytopenia	2 (9)	1 (2)
Gastrointestinal	1 (4)	2 (3)

*Values = No. of patients (%).

Toxicity

Leukopenia was the major toxic effect in both transitional cell carcinoma and renal cell carcinoma (table 1). There were five deaths prior to assessment for response. Two of the five fatalities were secondary to sepsis with grade 5 leukopenia. The other three were related to renal failure, disease progression, and septic shock. Severe to life-threatening leukopenia was observed in 16 of 79 patients. Moderate to severe thrombocytopenia occurred in six of 79 patients. Twenty of 79 patients had moderate to severe anemia during treatment courses and required transfusions. Progressive renal failure or blood loss was not present to explain the cause of anemia. Two patients developed severe back pain during infusion of VM-26, and both recovered when infusion was discontinued. Two patients had moderate hypotension during infusion, which disappeared after infusion was completed.

DISCUSSION

Responses seen with VM-26 in bladder cancer ranged from 5% (two partial responses in 40 patients) reported by Qazi et al (1) to 26.6% with two complete responses in another study (2). Yagoda (3) reported an overall response of 19% among 24 patients. In renal cell carcinoma, Pfiefle et al (4) reported three partial responses in 32 patients, whereas Hire et al (5) reported no responses in 13 patients.

In this study (SWOG 8101), one partial response was seen in renal cell carcinoma which lasted 6 months and one partial response was seen in transitional cell carcinoma of the bladder which lasted 5 months.

This study failed to show that VM-26 given weekly benefits patients with advanced renal cell carcinoma or transitional cell carcinoma of the bladder.

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Phase II Evaluation of Diaziquone in Pancreatic Carcinoma: A Southwest Oncology Group Study[1,2]

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Diaziquone (AZQ) is a rationally synthesized quinone with low ionization potential and good lipid solubility (1). The dose-limiting toxicity of AZQ in phase I clinical trials was primarily granulocytopenia and thrombocytopenia (2,3). A phase II trial was undertaken in an attempt to identify the potential antitumor activity of AZQ in metastatic or inoperable adenocarcinoma of the pancreas.

PATIENTS AND METHODS

Twenty-three patients with histologically proven disease were entered into the study. Study entry requirements included: age < 70 years; Southwest Oncology Group (SWOG) performance status ≤ 3; and life expectancy > 10 weeks. For patients who had received prior chemotherapy, at least 6 weeks had to have elapsed since the last dose of mitomycin and at least 3 weeks for doxorubicin or 5-fluorouracil before beginning AZQ therapy. Pretreatment leukocyte count > 4000/mm^3, platelet count > 100,000/mm^3, serum creatinine < 1.5 mg/dl, and BUN < 25 mg/dl were required. Patients who had had prior chemotherapy, were > 65 years of age, had bilirubin > 3 mg/dl, or had hepatic enzyme (AST, ALT) levels > 3 × the institutional normal were defined as poor-risk patients. All other patients were defined as good-risk patients. Good-risk patients started AZQ treatment at a dose of 40 mg/m^2 iv every 3 weeks. Poor-risk patients started treatment at a dose of 30 mg/m^2. The dose of AZQ was increased by 25% when nadir leukocyte and platelet counts exhibited tolerance (> 4000/mm^3 and > 100,000/mm^3, respectively) or decreased by 25% when nadir leukocyte and platelet counts were low (100,000/mm^3 or < 50,000/mm^3, respectively).

TABLE 1.—Patient characteristics*

Characteristic	
Entered	23
Evaluable	21
Prior chemotherapy	2
Median age in yrs (range)	55 (29–69)
Good-risk patients	57.5 (29–69)
Poor-risk patients	66 (51–69)
Male/female	17/4
Median SWOG performance status (range)	2 (0–3)
Median No. of courses (range)	2 (1–5)

*Unless stated otherwise, values = No. of patients.

Standard SWOG response and toxicity criteria were used.

RESULTS AND DISCUSSION

Twenty-one of the 23 patients registered were evaluable for toxicity and response (table 1). One patient refused therapy, and one was registered but not treated. Two patients received prior therapy with 5-fluorouracil, doxorubicin, and mitomycin. There were 16 good-risk and five poor-risk patients. Hematological toxicity was severe in the good-risk group, with five patients developing life-threatening toxic reactions. Ten patients had grade IV leukopenia, and seven patients had grade IV leukopenia and thrombocytopenia. It was not possible to determine whether anemia was secondary to treatment or disease. Nonhematological toxicity was greater in the

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good-risk group and consisted of nausea and vomiting (grade II), one patient with an anaphylactic reaction, and one patient each with grade I mucositis and diarrhea. At the time of this report, all patients had died. The median length of survival from registration was 18 months. There were no objective responses. We conclude that AZQ is inactive against adenocarcinoma of the pancreas.

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Etoposide, Ifosfamide, and Cisplatin in the Treatment of Advanced Non-Small Cell Lung Cancer[1]

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The combination of etoposide (VP-16) with cisplatin is widely used in advanced non-small cell lung cancer (NSCLC), with a mean response rate of 34% (1); this combination program is at least as active as the other most effective regimens in this neoplasm (2). Ifosfamide is reported to be the most active single agent (response rate, 26%) (3): this makes the drug suitable to be combined with cisplatin-based chemotherapy in NSCLC phase II trials in an attempt to improve the overall response rate.

PATIENTS AND METHODS

Forty-two patients with histologically proven NSCLC were entered in the study from December 1984 to October 1986. Protocol eligibility requirements were as follows: inoperable stage (American Joint Committee, 1973) IIIM0 and IIIM1 NSCLC (adenocarcinoma, squamous cell carcinoma, or large cell carcinoma), measurable or evaluable disease [World Health Organization (WHO) criteria], no previous chemotherapy and radiotherapy, performance status (PS) (ECOG scale) ≤ 2, and age < 70 years or age 70–75 years only if PS 0. Chemotherapy schedule was cisplatin at 60 mg/m^2 iv on Days 1 and 2, ifosfamide at 1200 mg/m^2 iv on Days 1-5 plus mesna at 480 mg/m^2 orally at time 0 and after 4 and 8 hours from ifosfamide administration, and VP-16 at 80 mg/m^2 iv on Days 1, 3, 5, and every 28 days until disease progression or to a maximum of six courses. Since the first 13 patients developed excessive myelotoxicity and nephrotoxicity, we decided to change the schedule, limiting the cisplatin dose from 60 mg/m^2 on Days 1 and 2 [high-dose cisplatin (HDP)] to 60 mg/m^2 on Day 1 only [low-dose cisplatin (LDP)]. Chemotherapy was delivered on an outpatient basis. One-week chemotherapy delay or standard dose reductions were applied in case of no full hematologic recovery. Patients were re-evaluated to assess objective response and treatment toxicity according to WHO criteria after the second course of treatment and then every two cycles.

RESULTS

All of the 42 eligible patients were considered for response, toxicity, and survival. Their characteristics are shown in table 1. Eight patients received only one cycle of chemotherapy due to early death in two patients, toxicity in two (gross hematuria and grade IV mucositis plus grade II nephrotoxicity, respectively), worsening of PS in one, patient refusal in one, and drug-related death in two (grade IV leukopenia + grade II nephrotoxicity and grade IV leukopenia + grade IV thrombocytopenia + grade III nephrotoxicity, respectively; both the patients had been treated with HDP): all these cases were considered as therapy failures. We observed three complete responses (CR) (one with HDP, two with LDP) and eight partial responses (PR) (six with HDP, two with LDP), with an overall response rate of 26.2% (95% confidence limits, 13.8%–42.0%); 18 patients had stable disease and 13 patients failed to respond. The overall median survival was 33 weeks. The median duration of response and responders' median survival were 40 and 62 weeks, respectively. Grade II–III nausea and vomiting and alopecia were present in most of the patients. Nine cases of severe leukopenia (including four grade IV, all treated with HDP) were noted, together with five cases of grade II–III anemia and three of thrombocytopenia (one grade IV with HDP). Grade III nephrotox-

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total entered	42
Median age in yrs (range): 61 (34-71)	
Sex	
Male	42
Female	0
PS	
0	9
1	25
2	8
Histology	
Adenocarcinoma	16
Squamous cell carcinoma	26
Stage	
M0	20
M1	22
Site of metastases	
Lung	8
Lymph nodes	6
Pleura	3
Brain	3
Liver	3
Adrenals	2
Bone	2
Others	2
Previous weight loss	
0	18
< 10%	12
> 10%	7
Unknown	5
Response to therapy	
CR	3
PR	8
Stable disease	18
Disease progression	13
Median response duration (wks): 40	
Median survival (wks): 33	

icity was reported in one patient (with HDP) and grade I–II was reported in four other patients (one with HDP, three with LDP). Two patients had gross hematuria, three had elevation of hepatic enzymes, and seven had mucositis. Paresthesias and ear loss or tinnitus were occasionally noted.

DISCUSSION

In our study the VP-16, ifosfamide, and cisplatin combination with cisplatin at 120 mg/m^2/course (HDP) showed severe toxicity. These toxicity data are in line with those by Loehrer et al (4) in germ cell tumors with a similar drug schedule. Reducing the cisplatin dose to 60 mg/m^2/course (LDP), the toxicity appeared acceptable. The overall response rate of 26.2% was not superior to that obtained with cisplatin and VP-16 combination therapy. In the few patients treated with HDP, an apparently good response (seven responses among 13 patients) was obtained at the expense of excessive toxicity. In conclusion, the combination of ifosfamide with VP-16 and LDP is reasonably well-tolerated and moderately effective in NSCLC but does not appear to offer any substantial advantage over the combination of VP-16 and cisplatin alone.

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Phase II Trial of Fludarabine Phosphate in Patients With Head and Neck Cancer: A Southwest Oncology Group Study[1,2]

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Fludarabine phosphate is a novel nucleotide synthesized specifically to resemble adenosine arabinoside but to have a slower rate of deamination. It has antitumor activity in animals and in vitro activity with established cell lines (1). In phase I trials in San Antonio, myelosuppression and thrombocytopenia were dose-limiting and allowed selection of a dose for phase II trials.

MATERIALS AND METHODS

Patients with advanced squamous cell carcinoma of the head and neck region (stage III or IV) were eligible for the study. Patient characteristics are shown in table 1. All patients had measurable disease, performance status of 2 or better by Southwest Oncology Group (SWOG) criteria, pretreatment BUN < 30 mg/dl, serum creatinine ≤ 1.5 mg/dl, wbc count $\geq 4000/\mu l$, and platelet count $\geq 125{,}000/\mu l$. All patients gave written, informed consent.

Fludarabine phosphate was given in 100 ml of normal saline iv over 30 minutes daily for 5 days. The starting dose for patients with prior radiotherapy was 18 mg/m^2 (90 mg/m^2/course) and for patients without prior therapy was 25 mg/m^2 (125 mg/m^2/course). Treatments were repeated every 28 days and the dose was increased by 25% if there was no hematologic toxicity (to a maximum of 28 or 31 mg/m^2 depending on prior treatment) or decreased by 25% if the wbc count nadir was $< 2000/\mu l$.

RESULTS

Twenty-five patients were entered in the study from 11 SWOG member institutions. All patients but one were fully evaluable for response and toxicity; the one inevaluable patient never received fludarabine phosphate. Three patients had received chemotherapy prior to or concurrently with radiotherapy and were free of disease (and chemotherapy) for at least 1 year. The 24 patients received a total of 66 cycles of fludarabine phosphate (median, two; range, one to ten).

Response criteria were those of SWOG. One partial response (60% shrinkage of neck nodes) was observed in a patient receiving the lower dose; response duration

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	25
Evaluable	24
Median age in yrs (range): 62 (45–74)	
Sex	
Male	22
Female	2
Prior therapy	
None	3
Radiotherapy	18
Chemotherapy/radiotherapy	3
Extent of disease	
Locoregional	11
Recurrence	8
Distant metastases	13
Recurrence	10
Lung involvement	9
Toxic effects	
Neutropenia	9
Grade 3	1
Grade 2	3
Thrombocytopenia (49,000 cells/mm^3)	1
Anemia	2
Hypotension, Grade 3	1
Nausea, Grade 1	4
Constipation, Grade 1	1
Muscle weakness, Grade 1	1

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was 11 months. An additional 11 patients initially had stable disease with treatment, while 12 patients had increasing disease during the first or second cycle. The median survival time for all patients was 24 weeks.

Toxicity was minimal. Lymphopenia was the most common toxic effect and was noted in 17 patients; seven had an absolute lymphocyte count $< 100/mm^3$. Four patients had somnolence, one moderate (possibly related to exhaustion and pain medications) and three mild. Two of these patients felt drowsy or slept more with each cycle of treatment. Other toxic effects are graded in table 1 by SWOG criteria.

DISCUSSION

The finding of lymphopenia as the most common side effect is consistent with the results of some phase I studies (1,2) but differs from other studies in which fludarabine phosphate was given every 12 hours or by continuous 5-day infusion (3–5), suggesting the possible importance of schedule and dose. Despite only mild toxicity, the finding of only one partial response demonstrated minimal therapeutic activity for fludarabine phosphate in the treatment of head and neck cancer.

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Prednimustine in Advanced Malignant Melanoma: A Phase II Study[1]

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In a phase II study, 22 patients with advanced malignant melanoma were treated with continuous prednimustine. A total of 59% (95% confidence limit, 36%–79%) experienced progressive disease, 36% (11%–50%) experienced no change, and 5% (0%–23%) experienced a partial remission lasting > 74 weeks.

Prednimustine is an ester of chlorambucil and prednisolone. Prednimustine is administered orally and is generally well tolerated. An antineoplastic effect of prednimustine in humans has been observed in non-Hodgkin's lymphomas (1,2), in chronic lymphatic leukemia (1,2), and in advanced breast cancer (3). In a phase II trial of the European Organization for Research and Treatment of Cancer (EORTC), one of eight patients with malignant melanoma experienced partial remission.

This study was conducted in order to confirm whether prednimustine is active in advanced malignant melanoma.

MATERIAL AND METHODS

The criteria of eligibility were: advanced measurable, evaluable, World Health Organization (WHO) (4) progressive malignant melanoma with no possible curative local treatment; between 18 and 70 years of age; and WHO performance status ≤ 2 (4). Patients with CNS disease, diabetes, or a history of gastric/duodenal ulcer were not included. The response to treatment was evaluated 4 and 8 weeks after initiation of therapy, and thereafter at intervals of 8 weeks. At toxicity, the dose was reduced according to WHO (4). Treatment was given until progressive disease or until prohibitive side effects occurred. Response to treatment was defined and measured according to the WHO criteria (4).

RESULTS

Twenty-two eligible and evaluable patients were registered. Patient characteristics before treatment, data regarding response to treatment, and side effects appear in Tables 1 and 2. Concerning the hematological toxic-

TABLE 1.—Patient characteristics and response

Characteristic	No. of patients
Entered	22
Median age at start of treatment in yrs (range): 51 (18–70)	
Median disease-free interval in mos (range): 18 (0–81)	
Sex	
Female	13
Male	9
WHO performance status at start of treatment	
0	14
1	5
2	3
3	0
4	0
Prior therapy	
Cytotoxic	0
Endocrine	0
Immunotherapy	2
Dominant site of disease	
Soft tissue	8
Cutaneous	5
Lymph nodes	5
Visceral	12
Liver	4
Lung	9
Bone	2
No. of recurrences	
1	7
2-3	9
> 3	6
Responses*	
Progressive disease	13 (59%)
No change	8 (36%)
Partial response	1 (5%)
Median time to treatment failure in wks (range): 7 (2- > 74)	
Median survival time in wks (range): 19 (2- > 74)	
Median treatment period in wks (range): 10 (2- > 74)	

* Progressive disease (95% confidence limit, 36%–79%); no change (95% confidence limit, 17%–59%); and partial response (95% confidence limit, 0%–23%).

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TABLE 2.—Toxic effects

Toxic effect	No. of patients (%)	95% confidence limits (%)
Hematological (WHO criteria)		
Grade 1	3 (14)	3–35
Grade 2	3 (14)	3–35
Grade 3	1 (5)	0–23
Grade 4	2 (9)	1–29
Nonhematological		
Nausea and vomiting (grade 1–2)	6	
Weakness	3	
Metallic taste	1	
Obstipation	1	
Dizziness	1	

ity, grade 3 toxicity appeared after 11 weeks of treatment, while grade 4 toxic effects appeared 23 and 24 weeks after start of treatment. Drug dose was correctly adjusted according to hematological values in 19 of 22 patients. Three patients did not receive the increased dose despite normal hematological values.

DISCUSSION

The results from the present phase II trial indicate that prednimustine has no significant activity in advanced malignant melanoma. The side effects in our study were moderate and were much less frequent than observed with the same schedule in a previous study of breast cancer patients (3). However, on the continuous schedule of prednimustine, hematological toxicity normally occurs after several months (J. Løber et al, unpublished results), and only a small fraction of the patients received treatment for that length of time, due to early progressive disease.

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European Organization for Research and Treatment of Cancer (EORTC) Phase II Study of Low-Dose Weekly Epirubicin in Metastatic Prostate Cancer[1,2,3]

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For the EORTC Genitourinary Tract Cancer Cooperative Group[9,10]

Few drugs have been evaluated in phase II studies in prostate cancer (1). Objective assessment of response is difficult and the European Organization for Research and Treatment of Cancer (EORTC) Genitourinary Group allows only bidimensionally measurable soft tissue or visceral lesions for this (2). Epirubicin was chosen for this study (activated in March 1984, closed in February 1985), since it was thought to be as active and less toxic than doxorubicin (3). Because of encouraging results using low-dose weekly doxorubicin in prostate cancer (4,5), epirubicin was given by weekly iv injection (12 mg/m^2; dose based on work in advanced breast cancer).

Entry criteria included: positive histology, any age, estimated survival < 90 days, indicator lesions as above, evidence of disease progression, normal blood cell count, adequate renal and liver functions, prior hormone therapy stopped at least 1 day, and effects of prior chemotherapy subsided. Previous anthracycline therapy, heart disease, other neoplasm, or inability to adhere to the weekly schedule precluded entry. Investigations including assessment of marker lesions according to World Health Organization (WHO) criteria were repeated every 4 weeks (blood cell counts weekly). Patients went off study for disease progression or any other reason in their best interest. Informed consent was required. Extramural review of the data was undertaken.

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Fully evaluable	33
Partly evaluable (toxicity)	35
Mean age in yrs (range): 66 (50–83)	
WHO performance status	
0	5 (15)
1	19 (58)
2	8 (24)
3	1 (3)
Prior therapy	
No prior endocrine therapy	7 (21)
Additive hormones	6 (18)
Orchidectomy	4 (12)
Orchidectomy + additive hormones	13 (39)
Orchidectomy + chemotherapy (estracyt)	1 (3)
Orchidectomy + hormones + chemotherapy	2 (6)
Nonhematologic toxicity (35 patients)	
Nausea only	7 (20)
Nausea and vomiting	7 (20)
Transient diarrhea	3 (9)
Alopecia	2 (6)
Lethargy	2 (6)
Phlebitis	1 (3)
Oral	1 (3)
Fever	1 (3)
Subcutaneous (rash)	1 (3)

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[2] Supported by Public Health Service grant CA-11488 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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[10] We thank Mrs. Anne Pickersgill for the preparation of the manuscript.

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TABLE 2.—Response (WHO)

	Indicator lesion					
	Lung	Nodes	Liver	Skin	Subcutaneous	Total (%)
CR	—	—	1	—	—	1 (3)
PR	—	2	1	—	—	3 (9)
No change	2	8	—	1	3	14 (42)
Disease progression	1	8	4	1	1	15 (46)
Total	3	18	6	2	4	33 (100)

Tables 1 and 2 give details of patients, responses, and toxicity. Hematologic toxicity was minimal: lowest wbc count was 2.7×10^9/L and lowest platelet count was 93×10^9/L. No patient went off study for toxicity but four refused further weekly injections. The mean number of cycles was 13 (range, four to 62), all at full dose.

Although one patient achieved complete response (CR) and two achieved partial response (PR) with marker lesion CR, the objective response rate (CR + PR) was only 12%. Good subjective improvement was seen in responders. This raises doubt that the dose intensity chosen was too low. Further studies employing more conventional doses and schedules are indicated.

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Phase II Study of Fludarabine Phosphate in Previously Untreated Patients With Colorectal Carcinoma: A Southwest Oncology Group Study[1,2,3]

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Fludarabine phosphate (FLAMP) is an analog of vidarabine which has shown preclinical antitumor activity (1-3). FLAMP was evaluated in a phase II study of previously untreated patients with advanced colorectal carcinoma.

All patients had measurable disease and normal hematologic, hepatic, and renal functions. No patients received other therapy while on protocol and all patients gave written informed consent according to institutional review board and Food and Drug Administration guidelines.

All patients were registered through the Southwest Oncology Group (SWOG) Statistical Office. FLAMP was administered iv as a rapid bolus infusion at a dose of 25 mg/m^2/day × 5 repeated every 28 days. Dose escalation to 31.25 mg/m^2/day was permitted, and dose decrease to 18.75 mg/m^2/day was done for unacceptable toxicity. Standard response and toxicity criteria, according to SWOG guidelines, were used to evaluate all patients.

Twenty-two patients were entered in this study. All patients were eligible for the study, with 20 patients being fully evaluable for response and 21 patients evaluable for toxicity. One patient died before receiving therapy. Patient characteristics, dose and number of courses of FLAMP, response, and toxic effects are shown in table 1.

A total of 65 courses of therapy were given. No responses to FLAMP in this dose or schedule were noted. Thirteen patients developed granulocytopenia, with a nadir granulocyte count of 1000/mm^3. Thrombocytopenia was infrequent. Seven patients developed somnolence during or shortly after FLAMP infusion. The

TABLE 1.—Patient characteristics*

Characteristic	
Entered	22
Eligible (males/females)	22 (12/10)
Evaluable for response/toxicity	20/21
Median age in yrs (range)	64 (37-78)
No. of courses evaluable	
18.75 mg/m^2/day × 5	11
25 mg/m^2/day × 5	41
31.25 mg/m^2/day × 5	11
40 mg/m^2/day × 5	2
Response	
Complete	0
Partial	0
No stable disease	10
Progressive disease	11
Early death	1
Toxic effects†	
Granulocytopenia (cells/mm^3)	
< 1500	8
< 1000	9
< 500	3
< 250	1
Thrombocytopenia (cells/mm^3)	
75,000-99,999	0
50,000-74,999	1
25,000-49,999	1
< 25,000	0
Somnolence	7
Anemia	5
Nausea and vomiting	3
Rash	1

*Unless otherwise specified, values = No. of patients.
†No. of patients with toxicity during treatment.

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somnolence was mild, self-limiting, and did not cause any patients to discontinue therapy. A mild anemia not requiring transfusion was seen in five patients. Nausea and vomiting was infrequent. One patient developed erythematous skin rash after 4 days of therapy, which resolved after discontinuation of FLAMP.

Since no antitumor responses were noted in this study, further use of FLAMP in the treatment of colorectal carcinoma using this dose and schedule are not warranted.

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Timing May be a Critical Factor in Drug Therapy

TABLE 1.—% of survivors 72 hours after a single iv injection of rHuTNF

Treatment group	Time of injection in HALO*						χ^2	P
	02	06	10	14	18	22		
% survival	90%	67%	10%	60%	90%	80%	20.2	< 0.01
No. of survivors/No. treated	9/10	6/9	1/10	6/10	9/10	8/10		

*HALO = hours after light onset (awakening corresponds to 12 HALO; rest onset to 0 HALO).

Recently Herxheimer (1) has pointed out that there are deficiencies in drug labeling. Another important piece of information that should be made available is the effect of *time of day of administration* on drug toxicity and efficacy. This is especially true for potent drugs such as cytotoxics and the newer genetically engineered biological response modifiers (BRMs).

Cisplatin, one of the most commonly used and broadly active chemotherapeutic agents, has been demonstrated to be minimally or markedly toxic, depending upon the time of day (circadian stage) that it is given (2). Circadian drug timing has been shown to be a critical determinant of organ-specific nonlethal kidney toxicity and in part determines the drug's ability to control cancer in human beings (3).

Many toxic, genetically engineered biological substances with anticancer activity are becoming available today. Because few, if any, biological processes do not participate in the ubiquitous high amplitude circadian rhythms, we have recently investigated whether the toxicity of recombinant human tumor necrosis factor (rHuTNF), a BRM, can be minimized by administration at an optimal circadian stage.

A single iv injection of rHuTNF at 750 or 1000 μg/kg given to 10-week-old female Balb/C mice was nearly ten times as toxic, in terms of survival, when administered midway in the usual daily waking span as when given just prior to usual awakening (table 1).

In order to optimize the selectivity of anticancer treatment we must maximize desired effects and minimize toxicity. Others have reported that several toxic effects caused by the first clinically available BRM, alpha interferon, are less severe in the evening (4).

Since submitting this letter we have repeated the toxicology study three times with precisely the same results. We have also found that the ability of rHuTNF to cure the Meth A sarcoma is dependent upon the time of day it is given to tumor-bearing mice. Sevenfold as many cures are seen if rHuTNF is given at a time of day associated with optimal tolerance (5). Chi-square analysis estimating the probability that the proportion of survivors was affected by the time of day of rHuTNF administration revealed a 99% probability that this effect was not random. These data indicate that the optimal therapeutic index of a new agent, rHuTNF, may depend heavily upon the time of day it is administered (6).

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Acute Laryngeal Edema After Single-Dose Irradiation and Doxorubicin

Cited below is a case report of acute laryngeal edema occurring immediately after ionizing radiation therapy in a patient who had received doxorubicin 3 weeks prior to the commencement of radiotherapy.

A 55-year-old female with documented metastatic breast carcinoma to bone received four courses of 5-fluorouracil, doxorubicin, and cyclophosphamide (FAC) by bolus injection through a left subclavian Port-A-Cath. Irradiation to the pelvis, lumbar spine, and hips was also administered without adverse side effects.

Three weeks after her last dose of doxorubicin (single dose, 65 mg; cumulative dose, 169 mg/m^2) she received 800 cGy in a single fraction through parallel opposed fields to the cervical spine for pain control. The single-dose fraction was chosen because of severe discomfort produced by mobilization. Fifteen minutes after irradiation, the patient noted increasing difficulty swallowing and breathing. Oxygen and dexamethasone (10 mg iv) were administered for mild edema of the oropharynx. A cardiorespiratory arrest occurred 60 minutes later. Marked edema of the oropharynx and epiglottis rendered intubation difficult. Erythema of the skin in the field of irradiation was noted. Following intubation, the patient was alert and responded to commands. She was extubated successfully after 12 hours. However, 12 hours later she sustained a second cardiorespiratory arrest from which she was not resuscitated.

The patient had no complaints referable to the head and neck and no soft tissue masses, thyromegaly, or lymphadenopathy had been noted. The patient's only medications were narcotic analgesics. The past history included no known allergies, mild hypertension controlled by diuretic, and two mastoid operations at the age of 20.

Postmortem examination revealed extensive bony and hepatic metastases. The oropharynx, larynx, bronchi, and esophagus were normal, and only mild edema and superficial tracheal ulcerations related to intubation were noted.

The potentiation of irradiation toxicity by doxorubicin has been reported, but reactions are usually not noted for approximately 7 days after radiotherapy (1), and acute reactions occurring within minutes of the irradiation have not been reported, to the best of our knowledge. The reaction mimicked a local acute allergic reaction, was devoid of systemic manifestations, and its temporal and topographical characteristics clearly relate it to the irradiation.

Although long-term control of bony metastases is most frequently attempted by using fractionated irradiation, single-dose and short fractionation palliative techniques are frequently utilized in some centers (2). Irradiation can produce acute edema when it is administered to other contained sites, such as the spinal cord or the brain. Few published data are available on morbidity, especially with the concurrent or antecedent use of anthracyclines. Of 12 patients treated by Pen (3) with single-dose fractions to the cervical spine, none developed acute complications.

Although our patient's susceptibility to acute laryngeal edema remains largely unexplained, hypersensitivity of the irradiated tissues to ionizing radiation must thus have been present to explain our patient's acute reaction. Doxorubicin is known to persist in tissues for long periods of time (4). Despite the lack of clinical evidence of impaired flow in the Port-A-Cath, it is possible that venous backflow did occur during drug administration, perhaps leading to higher tissue concentrations in the oropharyngeal area. One might postulate that persistent tissue-bound doxorubicin led to an increased sensitivity which resulted in acute cell damage with the release of vasoactive substances leading to edema. It is impossible to know whether a fractionated course would have produced the same toxicity. However, in view of the severity of the reaction, we recommend that single large doses of irradiation be used with caution in patients who have received doxorubicin and whose upper airway is included in the irradiation field. The same precautions would apply when other agents which are known to potentiate irradiation toxicity have been given prior to radiation therapy.

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Cyclophosphamide Hypersensitivity and Cross-Reactivity With Chlorambucil

A patient without previous exposure to cyclophosphamide developed urticaria after receiving cyclophosphamide as treatment for mycosis fungoides. Substitution with chlorambucil demonstrated cross-sensitivity. Such cross-sensitivity has not been previously reported. Literature is reviewed and underlying possible mechanisms are discussed.

Urticarial reactions to cyclophosphamide are rare. Cross-reactivity with chlorambucil has never been reported. We present a case of cyclophosphamide-induced and subsequent chlorambucil-induced urticaria in a 31-year-old woman with mycosis fungoides with no previous exposure to either drug.

CASE REPORT

The patient had pityriasis lichenoides from 9 years of age. In 1976 (at age 22), histiocytosis X was treated with vinblastine and prednisolone.

She first presented to our hospital in 1984 with an ulcerated nodular rash, biopsy of which showed dense infiltration of the dermis and blood vessel walls with atypical mononuclear cells, establishing the diagnosis of lymphomatoid papulosis. During the 9-year history of this rash, treatment with azathioprine, methotrexate, danazol, and dapsone had not provoked any allergic response. No topical alkylating agents had been given.

In 1986 a skin biopsy showing a monoclonal T-cell lymphocytic infiltrate demonstrated transformation to mycosis fungoides. Systemic chemotherapy was started with vincristine, 2 mg, cyclophosphamide, 850 mg iv, and oral prednisolone, 100 mg. Within 4 hours she developed diffuse, erythematous, pruritic wheals involving upper and lower limbs and trunk. There was no angioneurotic edema or anaphylaxis. The wheals settled promptly with iv hydrocortisone and promethazine; however, persisting diffuse pruritic erythema required repeated oral promethazine for 5 days before resolving.

Two weeks later she was given 100 mg of oral cyclophosphamide for new, painful, skin nodules typical of mycosis fungoides. Within 4 hours an urticarial eruption developed over her neck, trunk, arms, and thighs. Erythema and pruritus settled slowly over 7 days requiring repeated doses of iv hydrocortisone (100 mg) and promethazine (25 mg).

Peripheral blood examination showed no rise in serum uric acid, immunoglobulins, or eosinophil count. Renal function and liver function tests were normal. Biopsy of the skin rash showed a light perivascular inflammatory response consistent with urticaria.

Four weeks later, 18 hours after taking oral chlorambucil (15 mg) for worsening mycosis fungoides, she developed itchy, pruritic, erythematous eruptions over her breasts, back, and thighs. Intravenous ranitidine, 50 mg, had no effect, whereas 25 mg of iv promethazine caused prompt relief of itch and erythema. Repeated promethazine was needed for 2 days. Skin biopsy demonstrated a dense perivascular infiltrate consistent with urticaria.

Apart from metoclopramide and vincristine with the cyclophosphamide initially, no other medications were given. Previous metoclopramide had not caused any allergic reaction.

DISCUSSION

The severity of hypersensitivity reactions to cyclophosphamide has varied from mild pruritus (1) and "burning" of the tongue (2) to generalized urticaria and anaphylaxia (3,4). In most cases there has been prior exposure to cyclophosphamide and prompt response to administration of antihistamine and steroid. As in this case, hypersensitivity has occurred with both oral and iv cyclophosphamide (1,3), and mild initial reactions may become more severe with further exposure to cyclophosphamide, regardless of route of administration (1,3). In two patients with cyclophosphamide sensitivity who were later given chlorambucil, there was no cross-sensitivity (3,4). In one of the two cases of hypersensitivity to chlorambucil previously reported, an urticarial rash developed after 3 weeks of repeated exposure to chlorambucil (2).

The mechanism of hypersensitivity reactions to cyclophosphamide is unknown. An IgE-homocytotropic antibody binding cyclophosphamide was isolated from the serum of one patient who had suffered cyclophosphamide-induced urticaria, suggesting that an IgE-mediated type I anaphylactic reaction was involved (1). The lack of response to hydrocortisone and clear response to promethazine seen in this case further supports that IgE-mediated histamine release mediates this hypersensitivity reaction. Clear benefit from administration of promethazine as compared with ranitidine would suggest a role for H-1 histamine receptor stimulation in chlorambucil hypersensitivity.

A positive skin test response to phosphoramide mustard in the presence of a negative skin test response to cyclophosphamide was interpreted as indicating that hypersensitivity reactions may be due to a metabolite of cyclophosphamide (5). A lack of cross-sensitivity to other oral or parenteral alkylating agents in patients hypersensitive to cyclophosphamide is usual.

As in this case, hypersensitivity to cyclophosphamide and chlorambucil does not always require prior exposure. It is possible that prior chemotherapeutic agents can contribute to sensitivity to cyclophosphamide and chlorambucil, but the mechanism is unclear. In the presence of cyclophosphamide hypersensitivity, changing to another alkylating agent is still appropriate where

appropriate supervision of the patient would enable prompt treatment of hypersensitivity should this occur.

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Reversible Central Nervous System Toxicity Associated With High-Dose Chlorambucil in Autologous Bone Marrow Transplantation for Ovarian Carcinoma

In an effort to increase the response rate and survival in the patients with recurrent ovarian carcinoma, we devised a high-dose chemotherapy regimen (vinblastine plus etoposide plus chlorambucil) given in conjunction with autologous marrow rescue (BMT). We report three cases of significant, but reversible, neurologic toxicity induced by high-dose chlorambucil used in this setting.

Patient 1 was a 43-year-old woman who received as BMT cytoreduction iv vinblastine, 0.3 mg/kg/day, for 2 days, etoposide, 400 mg/m^2/day iv, for 3 days, and chlorambucil, 2 mg/kg/day orally, in three divided doses for 3 days. After 2.68 mg/kg total dose of chlorambucil she developed tremor of all extremities. An EEG showed bursts of delta activity in the frontal and central regions superimposed on symmetric well-modulated 8½ Hz background activity. Phenobarbital iv was started and she was continued on chlorambucil. After a total dose of 4 mg/kg of chlorambucil the patient became confused and agitated with visual, tactile, and auditory hallucinations. She had bilateral asterixis, generalized hyperactive deep tendon reflexes, and moderate myoclonus. Concurrent medication consisted of diphenhydramine, 200 mg/day, lorazepam, 8 mg/day, and droperidol, 2 mg/day. Electrolytes, glucose, calcium, magnesium, liver function tests, and arterial blood gases were all within normal limits. After 3 days the myoclonus cleared and after 9 days the mental aberrations resolved. A second EEG was normal. The patient reached complete remission clinically and biologically (normalization of Ca-125) but recurred 10 months later.

Patient 2 was a 43-year-old woman whose BMT regimen consisted only of 1 day of vinblastine and 2 days of etoposide (because of a reduced creatinine clearance) but unmodified dosage of oral chlorambucil. She developed myoclonus after 2.68 mg/kg of chlorambucil which worsened over the next 2 days but improved thereafter. The full dose (6 mg/kg) of chlorambucil could be administered, but she experienced transient hallucinations in the last day of drug administration, which cleared after 48 hours. The patient's Ca-125 levels normalized post BMT. A second identical BMT was performed 1 month later. This time after 3.34 mg/kg of chlorambucil she developed two episodes of generalized seizures (4 hours apart) followed by spontaneous and action-induced myoclonus. Concurrent medications were lorazepam, diphenhydramine, and droperidol (8, 200, and 2 mg/day, respectively). Electrolytes and all other nonhematologic laboratory values were within normal limits. The patient received iv phenobarbital and gradually improved over the next 3 days with a normal EEG at 24 hours. She was discharged on Day 19 with partial tumor response and no neurologic abnormalities.

Patient 3 was a 59-year-old woman who received the same BMT chemotherapy as Patient 2. After 4 mg/kg of chlorambucil she developed visual hallucinations and continual multifocal myoclonus. Concurrent medications were droperidol, 2 mg/day, and diphenhydramine, 300 mg/day. An EEG revealed slowing and disorganization of electrical activity with frontal spikes. The generalized myoclonus persisted for 7 days despite discontinuation of chlorambucil, diphenhydramine, and droperidol and correction of a transient hyponatremia. Repeat EEG 8 days later revealed slow, disorganized background activity without frontal sharp waves. Her post BMT course was otherwise uneventful, and she was discharged with full hematologic reconstitution. Only a partial remission was obtained, so 1 month later she returned for a second identical BMT course. While taking chlorambucil (after 4 mg/kg total dose) she again developed generalized myoclonus which resolved after 48 hours. An EEG revealed background slowing and intermittent rhythmic and polymorphic theta activity. Her second BMT course was complicated by *Streptococcus imitis* sepsis and multiorgan failure, resulting in death. Residual tumor was found in the abdominal cavity at autopsy.

Although chlorambucil-induced seizures have been previously reported in children (1,2), there are only three reported cases of seizures in adults receiving this drug. An elderly man with chronic lymphocytic leukemia developed three focal fits while receiving chlorambucil, 5 mg daily, for 3 weeks; this patient had epilepsia partialis continua of the right arm and flaccid paresis of right arm and right leg when he was rechallenged with chlorambucil. At this time, EEG showed continuous widespread theta activity with sharp waves but no focal features (3). The second case was an accidental overdose of multiple drugs including aspirin. This patient's clinical course was complicated with rhabdomyolysis, acute renal failure, and grand mal seizures (4). An elderly female with non-Hodgkin's lymphoma developed gait ataxia and generalized myoclonus after 2 days of 25 mg/day of chlorambucil with an EEG exhibiting sporadic widespread medium to high voltage, and slow wave activity in the theta and delta range with generalized frequent spikes (5). All three patients recovered without neurologic deficits. The mechanism of chlorambucil neurotoxicity has not been elucidated, but the spontaneous resolution of symptoms upon discontinuation of the drug suggests toxicity rather than a structural lesion. Because a portion of the drug has lipophilic properties, storage of the drug in fat may occur, explaining the prolonged toxic effect. One cannot rule out the additional neurotoxicity of the antiemetics used in these three patients. Diphenhydramine in daily doses > 300 mg may produce tremor, and lorazepam occasionally can produce visual hallucinations. Droperidol may induce extrapyramidal reactions including tremor of the limbs and restlessness. While practically all our patients exhibited clear-cut neurotoxicity in association with high-dose chlorambucil, no patient remained with mental or physical deficits as a direct effect of the high-dose chemotherapy. Most importantly, in one patient a 10-month complete remission could be obtained. Nevertheless, no more patients were entered on this protocol. To be successful, high-dose chemotherapy in conjunction with autologous BMT should be used much earlier in the course of the ovarian carcinoma disease, probably at the time of first recurrence.

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Rhabdomyolysis Associated With High-Dose Cytarabine

The use of high-dose cytarabine in the treatment of hematologic malignancies is widespread. Rhabdomyolysis, a syndrome known to have many etiologies, has not been described as a complication of chemotherapy with cytarabine. This report describes a case of non-oliguric rhabdomyolysis temporally related to the use of cytarabine.

CASE REPORT

A 44-year-old woman was diagnosed with acute myelomonocytic leukemia in June 1985. She was induced with doxorubicin and continuous infusion cytarabine. She relapsed in September 1986, and retreatment was begun with high-dose cytarabine, 3.6 g, infused over 75 minutes every 12 hours (2 g/m^2/twice a day). There was no family history of muscle disease or malignant hyperthermia.

One day following the initiation of chemotherapy (one dose) she became febrile to 40°C without a documented source of infection. Serum electrolytes including potassium and calcium, phosphate, BUN, and creatinine were within normal limits. Piperacillin and gentamicin were administered. The high-dose cytarabine was continued.

After six doses of cytarabine she remained febrile without shaking chills (39.8°C maximum temperature), and had an episode of ataxia, dysarthria, and lower extremity weakness, but without seizure activity. All symptoms resolved within 12 hours. A computerized tomographic head scan and lumbar puncture were unrevealing, and symptoms were attributed to high-dose cytarabine. Chemotherapy was stopped. At this time a CPK was 969 U/L. Aldolase and LDH were also elevated to 7.9 U/L (normal, 1.7–4.9 U/L) and 360 U/L

[normal (< 170)], respectively. The CPK continued to rise and peaked on Day 6 (> 10,000 U/L) following chemotherapy, returning to the normal range by Day 13. During this episode of rhabdomyolysis the urine output was adequate and myoglobin was present in the urine (after ammonium sulfate precipitation). The patient was vigorously hydrated, and the creatinine remained between 0.3 and 0.7 mg/dl.

During this time the serum potassium values ranged from 4.3 to 2.6 mEq/dl, due to a preexisting nephropathy and vigorous hydration. Phosphate levels were also low, ranging from 3.3 to 1.2 mg/dl (normal 2.5–4.5 mg/dl). Oral and iv potassium and phosphate were given.

The patient remained febrile, complaining of malaise, nausea, and mild diarrhea, but had no muscle pain, tenderness, or swelling. She had a positive *Clostridium difficile* titer and oral metronidazole was started concurrent with the resolution of rhabdomyolysis the patient defervesced. Cultures revealed no other pathogens and antibiotics were discontinued without incident.

DISCUSSION

Rhabdomyolysis is a clinical syndrome resulting from skeletal muscle injury with the release of muscle cell contents into the plasma. It is diagnosed by the increased serum levels of creatine kinase or aldolase, or by myoglobinuria. The patient described had two metabolic derangements occasionally associated with rhabdomyolysis: hypokalemia and hypophosphatemia. In addition, the patient was febrile, which has also been reported to cause rhabdomyolysis. However, due to the temporal association in this case we suggest that cytarabine was the causative agent of the rhabdomyolysis.

Classically, rhabdomyolysis causes hyperkalemia; however, hypokalemia has been reported in association with rhabdomyolysis (1). Hypokalemic rhabdomyolysis may be due to the inability of potassium-depleted muscle to respond to metabolic demands or hypoxia (2). However, rhabdomyolysis has not been reported with a potassium of > 2.0 mg/dl. Moreover, prior to starting therapy with cytarabine and at the onset of the rhabdomyolysis the potassium was within the normal range.

Similarly, hypophosphatemia has been reported in association with rhabdomyolysis. Hypophosphatemia may cause rhabdomyolysis by severe depletion of intracellular ATP (3). In this case the serum phosphate was normal before the onset of rhabdomyolysis, and found to be low 2 days after its onset, but was otherwise within normal limits and therefore an unlikely cause of the rhabdomyolysis.

Finally, during the 48 hours prior to the recognition of rhabdomyolysis, the patient was febrile with a peak temperature of 40.0°C but without shaking chills. Rhabdomyolysis has been reported with heat stroke, but usually with temperatures > 42.0°C (4).

We feel that this case of asymptomatic, nonoliguric rhabdomyolysis may be related to the administration of high-dose cytarabine. Haanen et al (5) have reported an alteration of mitochondrial staining kinetics in cells exposed to cytarabine. We suggest that a direct toxic effect of cytarabine on mitochondria may interfere with cellular energy metabolism. Such an effect may offer a mechanism for cytarabine-induced rhabdomyolysis. Physicians should be aware of this potential complication of high-dose cytarabine, as patients with malignancies may be predisposed to rhabdomyolysis because of concurrent fever, infections, or electrolyte imbalance.

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III Biennial Conference of the Indian Society of Oncology

Bangalore, India, February 10–12, 1988

For further information, contact: M. Krishna Bhargava, Director, Kimio and Chairman, Organizing Committee, Iso, Kidwai Memorial Institute of Oncology, Hosur Rd, Bangalore-560 029, India.

Clinical Hematology and Oncology: 1988

San Diego, California, February 15–17, 1988

For further information, contact: Bonny Mower, Department of Academic Affairs, Box 400S, Scripps Clinic and Research Foundation, 10666 N Torrey Pines Rd, La Jolla, CA 92037 (telephone: 619-457-8556).

Second International Congress on Neo-Adjuvant Chemotherapy

Paris, France, February 19–21, 1988

For further information, contact: Service d'Oncologie Médicale, Professeur Claude Jacquillat, 47, boulevard de l'Hôpital, 75651 Paris Cedex 13, France.

Third International Conference on Adjuvant Therapy of Breast Cancer

St. Gallen, Switzerland, March 3–5, 1988

For further information, contact: Prof. Dr. Hans-Jörg Senn, Oncology Center, Department of Medicine C, Kantonsspital, CH-900 F St. Gallen, Switzerland (telephone: 71-26-10-62).

Arizona Cancer Center 6th Winter Symposium: Advances in Hematologic Malignancies and Bone Marrow Transplantation

Snowbird, Utah, March 5–12, 1988

For further information, contact: Mary Humphrey, Conference Coordinator, Arizona Cancer Center, Tucson, AZ 85724 (telephone: 602-626-2276).

International Symposium Biotech Ria 88 on Molecular Probes: Technology and Medical Applications

Florence, Italy, April 11–13, 1988

For further information, contact: Fondazione Giovanni Lorenzini, Via Monte Napoleone, 23-20121 Milan, Italy (telephone: 02-702-267-783-868).

American Radium Society Seventieth Annual Meeting

Seattle, Washington, April 16–20, 1988

For further information, contact: Ms. Suzanne Bohn, Administrative Director, American Radium Society, 1101 Market St, 14th Floor, Philadelphia, PA 19107 (telephone: 215-574-3179).

8th Annual Meeting of the North American Hyperthermia Group

Philadelphia, Pennsylvania, April 16–21, 1988

For further information, contact: Radiation Research Society, 1101 Market St, 14th Floor, Philadelphia, PA 19107 (telephone: 215-574-3153).

36th Annual Meeting of the Radiation Research Society

Philadelphia, Pennsylvania, April 16–21, 1988

For further information, contact: Radiation Research Society, 1101 Market St, 14th Floor, Philadelphia, PA 19107 (telephone: 215-574-3153).

Fifth World Conference on Lung Cancer

Interlaken, Switzerland, August 28–September 1, 1988

For further information, contact: Rudolf A. Joss, MD, Secretary General, Institute for Medical Oncology, Inselspital, CH-3010 Bern, Switzerland.

2nd International Meeting of the Metastasis Research Society

Stadhalle, Heidelberg, Federal Republic of Germany, September 26–29, 1988

For further information, contact: Professor V. Schirrmacher, Institut für Immunologie und Genetik, Deutsches Krebforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg 1, Federal Republic of Germany.

Yearly Meeting of the German Institute for Laser Medicine

Vienna, Austria, October 5–8, 1988

For further information, contact: Doz. Dr. A. Tuchmann, c/o Wiener Medizinische Akademie, Alser Strasse 4, A-1090 Vienna, Austria (telephone: 0222-427165).

13th Congress of the European Society for Medical Oncology

Lugano, Switzerland, October 30–November 1, 1988

For further information, contact: F. Cavalli, MD, Division of Oncology, Ospedale San Giovanni, 6500 Bellinzona, Switzerland.

Workshop on Progress in Regional Cancer Therapy

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For further information, contact: Doz. Dr. R. Jakesz, Doz. Dr. H. Rainer, c/o B. Schick, Abteilung für Experimentelle Chirurgie der I. Chirurgischen Universitätsklinik, Alser Strasse 4, A-1090 Vienna, Austria (telephone: 0222-4800/2301).

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